Gender-Dependent Behavioral and Sensory Effects of a Commercial Mixture of Polychlorinated Biphenyls (Aroclor 1254) in Rats

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Developmental exposure to polychlorinated biphenyls (PCBs) has been associated with behavioral and cognitive deficits in humans and animal models. Perinatal exposure to PCBs has also been associated with sensory deficits in animal models. These effects were hypothesized to be mediated in part by ortho-substituted PCBs, which do not or weakly bind to the aryl hydrocarbon (Ah) receptor. The present studies were designed to determine whether perinatal exposure to Aroclor 1254, a commercial mixture of > 99% ortho-substituted PCBs, would affect cognitive and sensory function in Long-Evans rats. Adult male and female offspring of female rats fed Aroclor 1254 (Lot #124-191; doses of 0, 1, or 6 mg/kg/day; gestational day 6 through postnatal day 21; n = eight/group) were trained to perform a signal detection task capable of assessing sensory thresholds. Training included autoshaping and operant conditioning. Thresholds for detecting a 1-s light stimulus were determined under background illuminations ranging from 2 lux to complete darkness. Female rats exposed to Aroclor 1254 autoshaped more rapidly than control females, at a rate akin to control males. Control females had lower thresholds than control males at all levels of background illumination. These differences were abolished by Aroclor 1254, which reduced thresholds in males and increased thresholds in females. These data extend previous findings of gender-specific effects of PCBs on neurobehavioral development to measures of acquisition and sensory function.

Key Words: autoshaping; gender-dependent effects; Aroclor 1254; polychlorinated biphenyls; visual thresholds.

Polychlorinated biphenyls (PCBs) are a class of stable and heat-resistant halogenated aromatic hydrocarbons that were previously manufactured for many uses, including dielectric fluids, heat exchangers, hydraulic oil, paint additives, and plasticizers. Because of their extreme stability, these compounds have become ubiquitous contaminants of the biosphere (Tanabe, 1988), and their lipophilicity has facilitated their bioaccumulation in the food chain.

Observations of children exposed perinatally to PCBs suggest deficits in cognitive development. For example, studies of American children whose mothers consumed fish from Lake Ontario or Lake Michigan, which are contaminated with PCBs and other persistent pollutants, showed associations between maternal concentrations of PCBs during gestation and deficits in recognition memory (Fagan test) at 7 months of age (Jacobson et al., 1985), retarded physical growth and IQ (McCarthy scales) at 4 years of age (Jacobson and Jacobson, 1993), and persistent IQ deficits at 11 years of age (Jacobson and Jacobson, 1996). Prenatal PCB exposure also correlated with impaired short-term memory and reduced sustained attention (Jacobson et al., 1992), as well as with reduced cognitive function measured on the Kaufman Assessment Battery for Children (Patapho et al., 1999). These results suggest that PCBs may impair cognitive development of children when exposure occurs perinatally (i.e., in utero and during nursing); however, not all studies of children have found correlations between perinatal PCB exposure and adverse effects (e.g., Koopman-Esseboom et al., 1996; Rogan et al., 1987).

Studies of primates suggest a hypothesis that perinatal exposure to the PCB mixtures produces deficits in attention (Rice and Hayward, 1997; Schantz et al., 1991). For example, delay-independent deficits in delayed spatial alternation (DSA) were observed in monkeys exposed perinatally to Aroclor 1016 and 1248 (Schantz et al., 1991) or postnatally to a mixture designed to reflect the PCB congeners found in human milk (Rice and Hayward, 1997). Deficits in reversal learning (Schantz et al., 1991) may also be attributable to impaired attention (Sutherland and Mackintosh, 1970).

Sensory effects have also been associated with PCB poisoning. Developmental exposure to the coplanar congener 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) reduced a- and b-wave amplitudes of the scotopic electrotoretinogram (ERG) in female rats (Kremer et al., 1999). Developmental exposure to another...
copolanar, non-ortho-substituted PCB congener 3,3',4,4',5-
pentachlorobiphenyl (PCB 126) marginally elevated auditory
thresholds (Crofton and Rice, 1999), although no effect was
found on visual thresholds or visual evoked potentials in the
same rats (Geller et al., 2000). Aroclor 1254, a complex
mixture of mostly ortho-substituted PCB congeners, elevated
auditory thresholds to a greater extent than PCB 126 in adult
rats after developmental exposure (Goldey and Crofton, 1998).

Because of the findings suggestive of effects of developmen-
tal exposure to PCBs on attention and visual physiology, male
and female rats were tested with an operant signal detection
task, which can quantify visual sustained attention (Bushnell
et al., 1997). Aroclor 1254 was used in the present study as a
cost-effective representative of ortho-
substituted PCB congeners, elevated
visual thresholds, and electroretinograms. Rats were also tested
with the functional observation bat-
tery (F.O.B.) at four different ages,
motor activity (habituation), and sus-
tained attention, and were tested for
behavioral effects of pharmacologi-

cal challenge with haloperidal and
cocaine. These additional end points
will be reported on elsewhere.

MATERIALS AND METHODS

The procedures and results reported in this paper include autoshaping during
behavioral training, behavioral visual thresholds, and electroretinograms.

Other experiments and procedures carried out with these rats are enumerated in
Figure 1, and will be described elsewhere.

Animals. Two cohorts of pregnant Long-Evans hooded rats were obtained
6 weeks apart from Charles River Laboratory (Portage, MI) on gestational day
3 (GD 3; the day of insemination was GD 0) and housed in animal facilities
approved by the Association for Assessment and Accreditation of Laboratory
Animal Care (AAALAC). The animals were housed individually in standard
plastic hanging cages with heat-treated pine shavings as bedding. Food (Purina
lab chow) and water were provided ad libitum. Temperature and relative
humidity were maintained at 21 ± 2°C and 50 ± 10% respectively, with a 12-h
light/dark cycle. Light intensity in the animal colony ranged from 25 to 110 lux
depending on position on the cage rack. Animals from all dose groups were
evenly distributed among the shelves of the cage racks to ensure that there was
no bias due to light exposure conditions. All procedures were approved in
advance by the Institutional Animal Care and Use Committee of National
Health and Environmental Effects Research Laboratory of the U.S. Environ-
mental Protection Agency.

Dosing of animals. A commercial PCB mixture, Aroclor 1254 (Lot #
124-191; purity > 99%) was purchased from AccuStandard, Inc. (New Haven,
CT). The dosing solutions were prepared by dissolving this PCB mixture in
corn oil. The selected dosages were 0, 1, and 6 mg/kg/day, administered to the
dams by oral gavage (2 ml/kg) from gestational day (GD) 6 through postnatal
day (PND) 21, except on PND 1, when the dams were left undisturbed. The
dams were dosed once a day between 8:00 and 10:00 am and were weighed
every day before dosing.

Beginning on GD 22, rats were checked frequently for births, and the date
that birth was first discovered was assigned PND 0. All dams gave birth within
a few hours (> 90% of dams delivered successfully), and the litter size varied
from 4 to 17 pups. On PND 4, each litter was culled to four males and four
females. Three male and three female pups from each litter were randomly
selected for neurochemical analysis at different time points (to be reported on
elsewhere); the fourth male and female pup from each litter was used for
neurobehavioral assessment. Within treatment groups, pups were housed in
gender-matched pairs from PND 21 to PND 89.

Effects of dosing on dam and pup weights. Aroclor 1254 dosing did not
affect the dam body weight gain in either cohort. Total litter weight was
measured twice a week starting from PND 3 through weaning. There was a
slight decrease in body weight gain with Aroclor 1254 in one of the cohorts
(Mundy et al., 1998). Body weights of the two pups from each litter designated
for neurobehavioral assessment were recorded twice a week from PND 3
though adulthood as part of the weight maintenance necessary for behavioral
testing.

Procedures: Conditioned Behavior

Housing and weight maintenance. Beginning on PND 90, 35 male (18
from Cohort 1 and 17 from Cohort 2) and 34 female (18 from Cohort 1 and 16
from Cohort 2) rats were housed individually and placed on a restricted feeding schedule. A target body weight was calculated for each rat as 85% of its free-feeding weight. Mean (± SD) free-feeding weights were 462 ± 37 g for males and 259 ± 18 g for females; target body weights were 390 ± 22 g for males and 221 ± 12 g for females and were achieved over the course of a 2-week period of restricted feeding, with a minimum allotment of 5 g/day. After target weights were achieved, daily food rations were calculated according to an algorithm developed to maintain constant body weights (Ali et al., 1992). These weights (± 10 g) were maintained thereafter by scheduled feeding of rodent chow (Ralston Purina, St Louis, MO.) in the home cage after daily behavioral testing and on weekends. Water was supplied ad libitum in the home cage. All training and testing occurred during the light phase of the cycle. Sample sizes for the autoshaping studies were 12 for all groups except the 6 mg/kg dose, with males (n = 11) and females (n = 10). All groups were reduced in size to eight by random choice thereafter; in addition, one male rat from the 1.0 mg/kg dose group died of causes unrelated to treatment during visual discrimination training, leaving seven rats in that group.

**Behavioral training.** The rats were trained first to earn food by pressing a retractable response lever under an autoshaping-operant schedule, then to perform an operant task based upon the response rule: press one lever in the presence of a brief light flash (signal trial), and the other lever in its absence (blank trial). After reaching asymptotic accuracy, the rats then performed the task under conditions of varying signal intensity and background illumination. Rats from the two cohorts were trained and tested in the same apparatus during alternating periods of 1 to 3 months (Fig. 1, Acquisition 1 and 2), with Cohort 1 preceding Cohort 2 at each stage. While rats of one cohort were being tested, the other rats remained in their home cages under the weight maintenance conditions.

**Apparatus.** Four standard operant conditioning chambers (Model E1010, Coulbourn Instruments, Lehigh Valley, PA) were assembled as previously described for testing sustained attention (Bushnell et al., 1997; Bushnell and Rice, 1999). Each chamber was equipped with a food cup, two retractable response levers, an overhead house light with diffusing lens, a signal light of diameter 2.54 cm (backlit pigeon key, Coulbourn model E21-17, illuminated by an incandescent bulb behind a white plastic translucent diffuser), a loudspeaker, and a 4-kHz tone generator. The food cup was located in the center of the panel, 3.5 cm above the floor, with the two retractable levers on either side, 16 cm apart, 3.2 cm above the floor. The signal lever was assigned to the left lever in half the boxes and to the right lever in the other half; the opposite lever in each box was designated as the blank lever. In all boxes, the loudspeaker was located above the signal lever, at the top of the panel, and delivered masking white noise at 60 dB. The house light was mounted outside the test chamber, affixed to the ceiling of the sound-attenuating chamber. To facilitate training, the signal light was first located directly above the signal lever; it was moved to a position above the food cup, centered 19 cm above the floor of the chamber, after all rats achieved criterion accuracy (≥ 80% correct).

Signals were generated by adjusting the voltage of the signal lamp with a 256-step digital-to-analog converter (Coulbourn Model L65-28). A signal consisted of a 1-s increase in the brightness of the signal light to one of seven signal intensities above the ambient illumination of provided by the houselight. All light intensities were measured with a photometer (Model 450 with cosine probe, EGAndG, Inc., Salem, MA) centered on the floor of the test chamber, with the bulb continuously on (Bushnell et al., 1997). White noise in the chamber was set to 60 dBA with a sound level meter (Bruel and Kjaer model #2235). Signals were generated using SKED-11 software (State Systems, Kalamazoo, MI) running under RSX-11M plus on a PDP11/73 computer (Digital Equipment, Maynard, MA). The same software controlled all aspects of the behavioral testing.

**Autoshaping.** A combined autoshaping-operant procedure (Bushnell, 1988) was used to train the animals to press the lever for food (nutritionally complete 45-mg food pellets, PJ Noyes, Inc., Lancaster, NH). Each rat was acclimated to the test chamber and provided food pellets on a random time schedule with an average 32.5-s interpellet interval. Autoshaping began for each rat when it opened the food cup door to retrieve a pellet with a latency of less than 3 s on five consecutive trials of food pellet delivery. Each autoshaping trial began after an intertrial interval (ITI) averaging 17.5 s in duration (7.5 to 27.5 s). One lever was inserted into the chamber and the stimulus light adjacent to it was illuminated. After 15 s, the lever was retracted, the stimulus light extinguished, and a food pellet was delivered into the food cup. If the rat pressed the lever during its period of extension, the lever was immediately retracted, the stimulus light extinguished, and a food pellet was delivered. Four autoshaping sessions were conducted, each comprising 5 blocks of 10 trials, for a total of 20 blocks of trials.

**Absolute and increment threshold measurements.** Training steps that have been described previously for signal detection (Bushnell, 1997; Bushnell et al., 1994; Bushnell et al., 1997; Bushnell and Rice, 1999) were followed with the following changes: the opaque aluminum top and back of the internal test chamber were replaced with clear plastic; a new houselight was installed on the ceiling of the containment shell surrounding the test chamber; the interior of the chamber was painted white; a 4-kHz, 90-dB tone generator (Sonalert®) was installed in the location of the original house light; and trial-dependent changes in illumination were restricted to the signal light only. The visual cues previously used for reinforcement (Bushnell, 1997; Bushnell et al., 1994; Bushnell et al., 1997; Bushnell and Rice, 1999) were replaced by auditory cues (see trial description below). Training continued in both cohorts until each animal had achieved criterion accuracy of 80% correct (22 sessions).

Visual thresholds were determined under conditions of complete darkness in the test box, i.e., absolute threshold, and over 4 log units of background (conditioning) illumination in the test box, i.e., increment threshold, the just noticeable difference in intensity over a luminous background. Absolute threshold serves as a measurement of the neural noise present in the visual system; increment thresholds measure the ability of the visual system to adjust or adapt to changes in ambient lighting conditions.

Each daily session consisted of 300 consecutive trials. For analysis, each session was divided into three 100-trial blocks, each block lasting approximately 20 min. Signal and blank trials were presented in equal number in each session in a pseudorandom sequence. Signal intensities varied systematically among seven values during each block of each session. A single signal was presented on each signal trial after a constant ITI of 7 s; both levers were inserted 3 s after onset of the signal. These temporal parameters yielded an event rate of five trials per minute. Blank trials were presented identically, except the intensity of the signal light was not increased.

Background intensity was controlled with calibrated glass neutral density filters (Coherent-Ealing, MA). Signal intensity was determined by the combination of digital-to-analog voltage settings as detailed above and the interposition of neutral density filters (Wratten gel filters, Kodak, Rochester, NY) to shift the intensity range to that appropriate for the ambient illumination conditions. Rats were dark-adapted within the test room for a minimum of 45 min prior to testing.

Each trial began with both levers retracted from the chamber; both levers were inserted simultaneously at the end of the ITI. The levers were both retracted when one was pressed or if 5 s passed without a press. If no press occurred, a response failure was recorded and the trial was not repeated. Each correct response (a press on the signal lever on a signal trial, “hit”; or a press on the blank lever on a blank trial, “correct rejection”) was followed by a 200-ms, 4-kHz tone on every trial and delivery of a food pellet into the food cup on 80% of trials. After each incorrect response (a press on the signal lever on a blank trial, “false alarm”; or a press on the blank lever on a signal trial, “miss”), or response failure, the rat received a 15-dB increase in the white noise for 0.5 s during a 2-s period of timeout.

**Electroretinography.** After the completion of behavioral testing, retinal physiology was assessed with flash electroretinography. Rats were dark-adapted overnight. Under dim red illumination, each animal was anesthetized with a combination of ketamine (64 mg/kg) and xylazine (8 mg/kg), i.p. The animal was then placed in a stereotaxic device and the left eye was taped closed. The animal was placed on a heating pad and wrapped in a large Kimwipe to help maintain body heat, and a rectal temperature probe (YSI) was inserted.
The cornea of the right eye was anesthetized with 2 drops of proparacaine hydrochloride (0.5%) (Bausch and Lomb, Tampa, FL). The pupil was then dilated with 2 drops of tropicamide (0.75%), spaced 3 min apart, and 1 drop of phenylephrine hydrochloride (2.5%; Bausch and Lomb). Lubricant eye drops [Dextran 70 0.1%, hydroxypropyl methylcellulose 0.3% (Alcon, Inc., Puerto Rico)] were used to moisturize and lubricate the cornea.

The active electrode, a loop made of thin platinum wire (0.005 in.), was placed in light contact with the cornea. The reference and ground electrodes were subdermal needles inserted into the skin at the lateral canthus of the eye and in the skin of the neck, respectively. Responses were elicited with 10 µs flashes of white light from a Grass strobe (PS-22, Grass Instrument Company) projected onto the outside of a half table-tennis ball (Ganzfeld) used to diffuse the light and illuminate much of the visual field. The unattenuated luminance of the Ganzfeld was 10.62 cd/m^2 (Model 450 Radiometer/Photometer System, EG and G, Inc., Salem MA). The intensity of the light was attenuated using calibrated neutral density filters (Ealing, MA) in 0.5 or 1.0 log unit steps over a range of 6 log units. The interstimulus interval for the single flash ERGs was 45 s. After the final flash under dark-adapted conditions (0 attenuation), an adapting light of intensity 4.80 cd/m^2 was illuminated.

Stimuli were triggered and waveforms recorded using a system developed at the U.S. EPA for electrophysiological recording (Hamm et al., 2000). Signals were amplified 10,000 times and bandpass filtered (Neurodata 12 Amplifier, Grass Instrument Co.). On one channel, a bandpass of 0.3–1000 Hz was used to record the full ERG waveform; the bandpass was set to 100–1000 Hz on a second channel to isolate the oscillatory potentials (filter rolloff = 6 dB/octave). Single-flash ERGs and oscillatory potentials were recorded over a 300-ms epoch including a 20-ms baseline. A-wave amplitudes were measured from baseline to peak of the corneal negative deflection; b-waves were measured as a peak-to-peak measure from a-wave trough (baseline if no a-wave was present) to b-wave peak. Implicit times were measured from flash onset to the respective peaks. Oscillatory potentials (OPs) were examined from the brightest three dark-adapted flashes. Peaks OP1 through OP3 were examined. Amplitude was measured as the peak-to-peak difference between the OP peak and the negative trough immediately preceding it.

### Data Analysis

**Autoshaping.** The frequency of presses per block was recorded. The median number of lever presses per block was analyzed for each sex separately with categorical modeling (Proc CATMOD; SAS, 1990).

**Signal detection task.** For quantification of accuracy in the signal detection task, the number of hits, misses, correct rejections, and false alarms were recorded for each signal intensity during each session. The proportion of hits \( P(\text{hit}) = \frac{\text{(number of hits)}}{\text{(number of hits + number of misses)}} \) and the proportion of false alarms \( P(\text{fa}) = \frac{\text{(number of false alarms)}}{\text{(number of false alarms + number of correct rejections)}} \) were calculated for each signal intensity. \( P(\text{hit}) \) and \( P(\text{fa}) \) values were subjected to a repeated measures analysis of variance with Aroclor 1254 dose and cohort as between-group variables, gender nested within litter, and signal intensity and background intensity as repeated measures (Proc GLM, SAS, Inc., 1990). Huynh-Feldt degree-of-freedom (df) corrections were used to minimize the effects of asymmetrical variance-covariance matrices for repeated measures in all analyses. Statistical significance was tested with \( \alpha = 0.05 \) for each ANOVA.

**Absolute and increment threshold task.** First, the proportion of hits and the proportion of false alarms were calculated for each signal intensity during each session as detailed above. \( P(\text{hit}) \) values for each rat at each signal intensity were then corrected for “guessing” according to the following formula: \( P^*(\text{hit}) = \frac{P(\text{hit}) - P(\text{fa})}{[1 - P(\text{fa})]} \) (Green and Swets, 1974). This correction assumes that the true proportion of hits, \( P^*(\text{hit}) \), is elevated by an amount related to \( P(\text{fa}) \), and removes that contribution from the observed \( P(\text{hit}) \). Mean \( P^*(\text{hit}) \) for each stimulus intensity was calculated from the results of the two test sessions at each background intensity. Mean \( P^*(\text{hit}) \) as a function of signal intensity for each animal at each background condition was then fit with the following kinetic function adapted from visual electrophysiology (Birch, 1989):

\[
P^*(\text{hit}) = \left( I^* \left( P^*(\text{hit})_{\text{max}} \right) \right) / \left( I^* + \sigma^2 \right)
\]

where for a given \( P^*(\text{hit}) \) and intensity \( I \), the following three parameters are fit: \( P^*(\text{hit})_{\text{max}} = \) asymptotic maximum corrected hit rate; \( \sigma = \) semisaturation constant [intensity at \( \frac{1}{2} \) maximum \( P^*(\text{hit}) \)]; and the exponent \( n \), describing the slope of the function. \( \sigma \) reflects sensitivity and is used as used as an estimate of threshold intensity for each rat—a rightward shift (increasing \( \sigma \)) along the intensity axis indicates reduced sensitivity or increased threshold. Each fitted parameter was then analyzed in a nested model repeated measures ANOVA as described above. Data from five animals were eliminated from analysis of \( \sigma \) because their performance did not reach 50% \( P^*(\text{hit}) \) under all intensity conditions.

**Electroretinography.** Amplitude and implicit time for the a- and b-waves were analyzed with a nested model ANOVA as described above, with flash intensity as the repeated measures factor. Oscillatory potentials were similarly analyzed with each peak analyzed separately.

### RESULTS

**Autoshaping**

Most rats acquired the lever-press response under the contingencies of the autoshaping-operant schedule, and these proportions were approximately equal across treatment groups (Table 1). Analysis of the lever-press frequencies was conducted on the first 12 blocks of trials because response patterns for all animals had reached stability by that stage of training. Inspection of the data revealed that the rates of acquisition of the response (slope of the curves) did not differ across groups, but the number of trial blocks administered before pressing began varied widely (Fig. 2). Categorical modeling analysis...
showed that for males, perinatal exposure to Aroclor retarded the onset of lever pressing, whereas for females, perinatal exposure to Aroclor accelerated it. The effect in the females was PCB dose dependent, whereas in the males it was not. The acquisition curve for the high-dose females lay very close to that of the control males, and the curve for the low-dose males fell close to that of the control females (Fig. 2).

Psychophysical Estimates of Absolute and Increment Thresholds

The rats generated frequency-of-seeing functions with $P^s$(hit) values spanning the range from close to 0% correct for the dimmest stimuli to asymptotic performance over 90% correct for the brightest stimuli at each background condition (Fig. 3). Within-subject analyses yielded strongly significant effects ($p < 0.0001$) for the intensity of the detection stimulus and the background illumination, showing that the animals responded as expected to experimental manipulations, i.e., $P$(hit) increased with detection stimulus intensity and the entire function shifted as expected with background intensity. There were no significant effects of dose or sex on false alarm rate. There was an effect of background intensity, but this was likely an artifact of the detection stimulus range used in each background condition. Specifically, under conditions where fewer stimuli were detected, e.g., the $2.00 \times 10^{-3}$ condition, there were a greater number of false alarms, i.e., the rats were guessing more.

Exposure to Aroclor 1254 tended to shift the psychometric functions to the right for females and to the left for males (Figs. 3A and 3B). This pattern was confirmed by the ANOVA of the $P^s$(hit) data, which yielded a significant dose by sex by background interaction $[F(12,84) = 3.24, p < 0.0007]$. The rightward shift in the curves from the dosed females denotes a decrease in sensitivity relative to the control females, and the leftward shift in the functions from the dosed males denotes increased sensitivity in the dosed males relative to the control males. In stepdown analyses of these data by sex, the effect of Aroclor 1254 was significant in males ($F(2,20) = 3.69, p < 0.043$) but not in females. Some effects of cohort were noted, but these did not interact with either dose or sex effects.

Analysis of the parameters derived from the analytic fits to the data, in particular the threshold parameter $\sigma$, help to clarify the effect of dosing on increment threshold performance. For these fits, $\sigma$ is the intensity at $\frac{1}{2}$ maximal $P^s$(hit). $\sigma$ can serve as an estimate of threshold because it marks the position of the curve along the intensity axis. Figure 4 shows the values of $\sigma$ as a function of background illumination intensity, i.e., threshold versus intensity function (TVI). The TVI measures the ability of the visual system to adjust its sensitivity relative to
the level of background intensity, a process known as light adaptation. Absolute threshold, estimated from psychophysical performance on the task with no background illumination, appears at the far left of each plot. The horizontal line through the “dark” condition illustrates the asymptotic detection characteristic of luminance threshold measurements (c.f. Hood and Finkelstein, 1988). The points to the right of absolute threshold mark the increment threshold, that is, the detection stimulus intensity threshold in the presence of background illumination. The linear increase of threshold with background intensity is characteristic of Weber’s Law, and again shows that the animals were performing as expected.

Analysis of the threshold parameter \( \sigma \) yielded an overall dose by sex interaction \( [F(2,16) = 3.62, p < 0.05] \), similar to the interaction yielded by the analysis of the complete psychometric functions. There were no overall or interaction effects of cohort on \( \sigma \); subsequent analyses of \( \sigma \) were done without cohort as a factor. Further, there were no significant differences between the slopes of the ascending limbs of the TVI curves as a function of dose, sex, or cohort.

Stepdown analyses of each dose group separately help explain the dose*sex interaction. In the control group, there was a significant effect of sex on \( \sigma \), with females more sensitive than males (Fig. 4, left) Univariate statistics show that this difference was significant at 4 of 5 background conditions tested. In the low-dose group and high-dose groups, the threshold versus background intensity functions from males and females did not differ significantly (Fig. 4, center and right).

Electroretinogram

Electroretinograms recorded from dark-adapted rats yielded waveforms that showed the expected increases in a- and b-wave amplitude and decreases in implicit time with increasing intensity, but showed no significant effects of dosing with Aroclor 1254. A-wave parameters were not affected by sex and/or treatment with Aroclor 1254. B-wave (peak-to-peak) amplitude increased with intensity, whereas implicit time decreased over the intensity range tested. Females had greater b-wave amplitudes and implicit times than males across intensities; b-wave amplitude in the high-dose males was somewhat reduced (Fig. 5). Some effects of cohort were noted, but these did not interact with either dose or sex effects.

OPs from the highest three intensities tested were also examined. OP1, OP2, and OP3 amplitude increased, whereas latencies decreased with increasing intensity. Treatment with Aroclor 1254 produced dose-related trends toward increased OP1, OP2, and OP3 latency and decreased OP1, OP2, and OP3 amplitude, but with the exception of OP2 amplitude \( [F(2,18) = 3.57, p < 0.05] \), these effects fell short of statistical signifi-
DISCUSSION

Significant gender-related effects of Aroclor 1254 were found on measures of behavior and sensory function reported in this paper. Although our initial hypotheses involved potential effects of dosing on attentional and sensory processes, the major effects noted were gender-specific alterations in two sexually dimorphic tasks, autoshaping and psychophysical luminance threshold determination. Developmental exposure to Aroclor 1254 eliminated the gender-related differences present in adult rats tested one and a half years after exposure (Fig. 1).

To the best of our knowledge, this is the first report of feminizing effects following exposure to an Aroclor mixture in a nonsexual end point. In a third measure, the dark-adapted ERG, b-wave amplitudes and latencies from female rats were larger than those from males, but developmental exposure to Aroclor 1254 had no effect on this difference.

Demasculinization and feminization of both sexual (Bjerke et al., 1994; Bjerke and Peterson, 1994; Mably et al., 1992) and nonsexual development and behaviors have been reported in conjunction with developmental exposure to PCBs, dioxins, and their contaminants. Children of women exposed to PCBs and polychlorinated dibenzofurans (PCDF) in the Yu-Cheng, Taiwan, accidental poisoning were evaluated with a test of spatial ability on which males ordinarily outperform females (Guo et al., 1995). The developmentally exposed male children performed more poorly than male controls and similarly to the females. There was no effect on females.

In animal models, developmental exposure to a mixture of 14 congeners present in breast milk resulted in the feminization of male rat behavior in a sexually dimorphic sweetness preference test along with decreases in testosterone levels, testes weights, and aromatase activity (Hany et al., 1999). Male rats have also been shown to be selectively affected by perinatal PCB exposure. Adult female offspring of rats dosed with PCB 28, PCB 118, or PCB 153, all ortho-substituted congeners, were slower to acquire a T-maze delayed spatial alternation task (Schantz et al., 1995). The Aroclor 1254 used to treat the animals in this study contained >99% ortho-substituted PCBs by weight (Frame et al., 1996; Kodavanti et al., 1999). Although it is possible that the alterations in performance identified in this report are due to dioxin toxic equivalence (toxic equivalency quotient = 38.05 μg/g), these findings lend support to the structure-activity models determined in vitro that suggest that many ortho-substituted PCBs have neurotoxic potential (Kodavanti and Tilson, 1997; Kodavanti et al., 1996).

In autoshaping, the initial stage of operand training, the onset of bar-pressing is generally faster for males than females (Van Haaren et al., 1987). Perinatal exposure to Aroclor 1254 retarded the onset of bar-pressing among the male rats such that performance was close to that of the control females, whereas exposure accelerated bar-pressing among females such that the rate of acquisition of the bar-pressing among the high-dose group was close to that of the control males. These results suggest that the observed alterations in behavior are due to feminizing and masculinizing effects of the exposure to Aroclor 1254 on males and females, as discussed below. We note, however, that the gender-dependent effects of Aroclor 1254 on autoshaping are not easily explained by current knowledge of gender differences in behavior during autoshaping. Physical size and strength may facilitate autoshaping in males, because lever-pressing follows initial exploration of the lever as a signal for reinforcement (Hearst and Jenkins, 1974; Jenkins and Moore, 1973), and more robust exploration of the lever will engender detectable responses (presses) sooner than will more delicate exploration. However, the body weights of neither the males nor females in this study were affected significantly by Aroclor treatment, and the males were maintained about 200 g heavier than the females throughout the study. Males also tend to engage more actively in lever-pressing behavior than females in several contexts. In discriminated autoshaping, in which one lever is paired with food (CS+) and a second lever is not (CS–), male rats pressed both levers more than females, either during initial training, or after reversal of the contingencies (Van Haaren et al., 1987). Similar sex differences have been reported for other operand schedules, including differential reinforcement of high-rate (Van Haaren et al., 1986) and low-rate responding (van Hest et al., 1987a), as well as lever-holding behavior (van Hest et al., 1987b). The neurobiological basis for this gender difference has not been determined.

The lack of effects of Aroclor 1254 on an attentional task performed by the same rats suggests that the effects on the perceptual task are due to sensory rather than cognitive effects (Bushnell et al., 1999). The primary evidence for this conclusion involves the pattern of effects on P*(hit) and P(fa). A horizontal shift in the P*(hit) by intensity gradient, in the absence of a change in P(fa), indicates a change in visual threshold (Bushnell et al., 1997; Bushnell et al., 1998; Geller et al., 2000). A major component of the sustained attention task is determined by visual perception. In these animals, however, although there were Aroclor-induced shifts in sensory thresholds, these were not sufficiently large to impair the perception and associative processing that the attention task demands.

In the behavioral assessment of visual thresholds, the control females were more sensitive than males under all test conditions; they needed less light to perform at criterion level under absolute and increment threshold conditions. Developmental treatment with Aroclor 1254 made the male rats more sensitive, i.e., more like the females. There was also a trend toward the dosed females being less sensitive, i.e., more like the males. These changes in threshold associated with dosing effectively eliminated the gender effect on visual thresholds. These changes were evident in shifts in the psychometric and TVI functions (Figs. 2 and 3).
The source of the exposure-related differences in sensitivity is not easily identified. The marginal dose by sex effect on the flash ERG, in the direction opposite that of the behavioral changes, i.e., males show increased sensitivity behaviorally but reduced ERG amplitudes, suggests that the effect is not on the photoreceptors or bipolar/Müller cells of the retina. One explanation for altered sensory thresholds may be that hypothyroidism associated with dosing with Aroclor 1254 led to alterations in the ganglion cell layer of the retina, as it did in propylthiouracil-treated rats (Leung et al., 1992; Navegantes et al., 1996). A change in ganglion cell neurons would not be reflected in the standard dark-adapted ERG. One could also speculate that recently identified estrogen receptors in the retina, located largely in the photoreceptor layer, may play a role in retinal growth and differentiation during development (Kobayashi et al., 1998; Ogueta et al., 1999), and that postulated estrogenic or antiandrogenic effects of exposure to Aroclor 1254 (Hany et al., 1999; Sager and Girard, 1994) altered retinal physiology. Another possibility is that subtle changes in the physiology of the visual cortex suggested by PCB-driven alterations in visual cortex long-term potentiation (Altmann et al., 1998) are expressed as changes in visual threshold. The visual cortex is known to be sexually dimorphic in Long-Evans rats, and dendritic organization in visual cortex has been shown to be more sensitive to manipulation by external stimuli in males than in females (Juraska, 1984; Seymour and Juraska, 1997).

Although gender-specific behavioral effects have not been linked to Aroclor 1254 prior to this report, developmental exposure of males has been found to have antiandrogenic effects, reducing testosterone levels and testes weights (Hany et al., 1999) and altering levels of testosterone hydroxylases and androstenedione formation (Haake-McMillan and Safe, 1991). In females, effects of exposure to Aroclor 1254 include delays in puberty, decreased uterine response to estrogens stimulation, impairment of fertility, and irregular cycle patterns, all of which are consistent with interference with the estrogenic system (Krishnan and Safe, 1993; Sager and Girard, 1994). Masculinization of females may be due to the hydroxylated metabolites of the non-coplanar PCBs in Aroclor 1254, as these metabolites have been shown to inhibit estradiol sulfotransferases and to bind the estrogen receptor (Connor et al., 1997; Kester et al., 2000).

In summary, these results illustrate gender-specific effects of perinatal dosing with Aroclor 1254, a largely ortho-substituted PCB mixture, on measures of learning and sensory function, tested approximately 3 months and 18 months after the cessation of dosing. The persistence of effects will after dosing and the presence of effects in both young and mature adults suggest that neural development was affected in these animals.

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