Gestational-Lactational Exposure to Aroclor 1254 Impairs Radial-Arm Maze Performance in Male Rats

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Developmental exposure to polychlorinated biphenyls (PCBs) has been associated with cognitive deficits in children. The current study assessed effects of gestational and lactational exposure to a commercial PCB mixture, Aroclor 1254 (A1254), on spatial learning and memory in rats, using the radial-arm maze (RAM). Pregnant Long-Evans females (10/dose group) were exposed to 0 or 6-mg/kg/day A1254 (po in corn oil) from gestation day (GD) 6 to weaning at postnatal day (PND) 21. After they reached adulthood, 1 male and 1 female from each litter were tested on a working/reference memory task using a 12-arm RAM. Eight of the 12 arms were baited, with the pattern of baited arms remaining the same on every trial for each rat. Compared to control males, the A1254-exposed males made significantly more working memory errors (2.15 ± 0.13 and 3.20 ± 0.18 errors ± SEM for control and A1254 males, respectively) and reference memory errors (3.17 ± 0.10 and 4.13 ± 0.14 errors ± SEM for control and A1254 males, respectively) on the RAM. In contrast, A1254-exposed females were not impaired relative to control females on the RAM. Drug challenges with dizocilpine (MK-801) and scopolamine did not differentially affect working or reference memory of control and exposed rats. These data suggest that perinatal exposure to A1254 may cause sex-specific deficits in spatial learning and memory, and that NMDA-mediated and muscarinic neurotransmission, as assessed with the drug challenges, were not markedly impaired in the A1254-exposed animals.

Key Words: PCB; Aroclor 1254; gestational-lactational exposure; spatial learning and memory; radial-arm maze; rats; drug challenges.

Polychlorinated biphenyls (PCBs) are persistent and ubiquitous environmental contaminants that were commercially produced from 1929 until the late 1970s (Tanabe, 1988). Their primary use was as dielectric fluids for transformers and capacitors, but PCBs were also used as hydraulic lubricants, heat-transfer fluids, flame retardants, sealants, plasticizers, pesticide extenders, and in carbonless copy paper (Safe, 1994). Due to their high lipophilicity and low biodegradability, PCBs from the environment bioaccumulate in the adipose tissue of living organisms, including humans. PCBs stored in maternal body fat are transferred from mother to offspring via both placental and mammary passage (Bush et al., 1984; Jacobson et al., 1984; Lanting et al., 1998; Patandin et al., 1997).

Some, but not all, epidemiological studies have documented lasting cognitive deficits in children exposed to PCBs during development. Accidental contamination of rice oil in Japan in 1968 and Taiwan in 1979 exposed children to both PCBs and polychlorinated dibenzofurans. Compared to matched controls, exposed children in Taiwan scored lower on standardized intelligence tests (Chen et al., 1992). The cognitive effects of developmental PCB exposure have also been assessed in children exposed to PCBs through maternal consumption of sport-caught Lake Michigan fish (Jacobson et al., 1986, 1983). Decrement in cognitive function were observed in infancy (Jacobson et al., 1985), and persisted at 4 (Jacobson et al., 1990, 1992) and 11 (Jacobson and Jacobson, 1996) years of age. A study in North Carolina did not find any persistent cognitive deficits associated with prenatal PCB exposure from background environmental sources (Gladen and Rogan, 1991). However, a similar study conducted in the Netherlands did find that prenatal exposure to background environmental PCBs was correlated with poorer cognitive abilities at 3.5 years of age (Patandin et al., 1999).

The relationship between PCB exposure and cognitive function in these epidemiological studies is by necessity correlational. However, laboratory studies in animal models confirm that developmental exposure to PCBs can result in persistent cognitive deficits. Adult rhesus monkeys exposed to commercial mixtures of PCBs (Aroclor 1016 or 1248) via maternal transfer during gestation and lactation were impaired on learning and memory tasks, such as spatial discrimination reversal.
learning, and delayed spatial alternation (DSA) (Bowman et al., 1978; Levin et al., 1988; Schantz et al., 1991). Rice and Hayward (1997) also reported impaired performance on a DSA task in adult monkeys exposed from 0 to 20 weeks-of-age to a mixture of PCB congeners similar to that found in human breast milk. Rice also observed deficits on fixed interval and differential reinforcement of low-rate operant tasks in the postnatally exposed monkeys (Rice, 1997, 1998).

Rodent studies have also documented impairments on retention of visual discrimination (Lilienthal and Winneke, 1991), water-filled multiple T-maze learning (Shiota, 1976), and active avoidance learning (Lilienthal & Winneke, 1991; Panteleoni et al., 1988; Storm et al., 1981) after prenatal and/or lactational exposure to commercial PCB mixtures. A study in which Sprague-Dawley rats were exposed to individual ortho-substituted PCB congeners (PCB 28, 118, or 153) via maternal exposure on gestation day (GD) 10–16 found impairments on the DSA task in adulthood, but only in female rats (Schantz et al., 1995). Learning of the 8-arm radial-arm maze (RAM) was not affected in male or female rats (Schantz et al., 1995). In contrast, offspring of Sprague-Dawley rats exposed to coplanar PCB congeners on GD 10–16 were not impaired on either DSA or the 8-arm RAM (Schantz et al., 1996). Rice and colleagues also failed to find impairments on DSA (Rice, 1999) or on a number of other tests of learning and attention in offspring of Long-Evans rats exposed to coplanar PCB 126 throughout gestation and lactation (Bushnell and Rice, 1999; Rice and Hayward, 1998, 1999).

The underlying neurophysiological changes responsible for the neurobehavioral effects of PCBs are not well understood, but evidence suggests that both commercial PCB mixtures and individual ortho-substituted PCB congeners impair hippocampal long-potentiation (LTP) (Gilbert and Crofton, 1999; Gilbert and Liang, 1998; Niemi et al., 1998; Wong et al., 1997; Gilbert et al., submitted). LTP is a physiological phenomenon, which is widely accepted as a neural substrate of memory storage (Martinez and Derrick, 1996; Morris & Frey, 1997). Thus, disruption of LTP could explain the learning and memory impairments observed in both humans and animals following developmental exposure to PCBs.

Although studies of specific PCB congeners (e.g., Bushnell and Rice, 1999; Rice, 1999; Rice and Hayward, 1998, 1999; Schantz et al., 1995, 1996) provide valuable information for their individual and subgroup characterizations, humans are exposed to complex mixtures of PCBs. Exposing laboratory animals to commercial PCB mixtures is an economical and practical method of simulating human exposure. PCBs in the environment originate primarily from commercially produced mixtures, and the more highly chlorinated PCB congeners tend to be the most biologically stable. Aroclor 1254 (A1254), one of the more highly chlorinated commercial PCB mixtures, most closely represents the congeners that dominate in environmental samples (Hansen, 1999). Thus, it is relevant to use A1254 in animal studies of neurobehavior following developmental exposure.

In the current study, spatial learning and memory were assessed in adult Long-Evans rats gestationally and lactationally exposed to the A1254. The RAM was selected because it is a hippocampally mediated task (Olton et al., 1979) and because some investigators have reported a link between RAM learning and hippocampal LTP (e.g., Balschun et al., 1999; Butelman, 1990; Ishihara et al., 1997). Drug challenges with dizocilpine (MK-801), an N-methyl-D-aspartate (NMDA) antagonist, and scopolamine, a muscarinic antagonist, were used to investigate the potential involvement of the cholinergic and glutamatergic neurotransmitter systems in mediating effects of PCB exposure. NMDA receptors have been implicated in spatial learning and are critical in the induction of certain types of LTP (Collingridge and Bliss, 1995; Morris et al., 1986). The cholinergic system also plays a vital role in spatial memory (Stancampiano et al., 1999; Winkler et al., 1995), and hippocampal cholinergic function has been shown to be altered by early PCB exposure (Eriksson and Fredriksson, 1998; Juarez de Ku et al., 1994). A companion study (Gilbert et al., 2000) evaluated spatial learning in the Morris water maze and hippocampal LTP in littermates of some of the rats tested in this study.

**MATERIALS AND METHODS**

**Animals and exposure.** Sixty-seven primiparous Long-Evans female rats were obtained from Charles River (Raleigh, NC) on GD 2, in 2 cohorts spaced 8 weeks apart. Dams were housed individually in standard plastic hanging cages (45 × 24 × 20 cm) with sterilized pine shavings as bedding. Animals were maintained in temperature- and humidity-controlled rooms (22 ± 2°C, 40 ± 20% humidity) on a 12-h light-dark schedule (lights on at 0600). All females were weighed on GD 5 and were assigned to treatment groups by balancing for body weight.

Previous studies (Goldey et al., 1995; Goldey and Crofton, 1998) have shown A1254 doses ≥ 8 mg/kg/day from GD 6-PND 21 caused significant and persistent deficits in pup body weight from birth to adulthood, and 50% pup mortality by PND 21. The 6.0-mg/kg/day dose of A1254, used in this study from GD 6 to weaning, was selected because previous studies showed that it eliminated the postnatal mortality associated with higher doses of A1254 and produced only a transient (10–15%) decrease in postnatal body weight gain that recovered by weaning (cf., Crofton et al., 2000). This treatment regimen also produced a transient hypothyroxinemia (Crofton et al., 2000).

The dams were administered 0 or 6 mg/kg A1254 (AccuStandard, Lot # 124–191) dissolved in corn oil, via gavage for 7 days/week from GD 6 to postnatal day (PND) 21. A1254 Lot #124–191 has a similar congener profile to Lot G4, described in Frame et al. (1996). Dams were weighed daily to calculate doses. Litters were culled to 8 pups on PND 4 and weaned on PND 21. For the first week following weaning, each litter was separated by sex. At PND 28, the pups were housed in same-exposure, single-sex pairs. Ten litters/exposure group were randomly selected, and 1 male and 1 female offspring from each of those litters were selected for RAM testing. As reported in the companion paper (Gilbert et al., 2000), an additional 1 male and 1 female from each litter were retained for the tests of Morris water maze learning, and 1 male from each litter was used for the studies of hippocampal LTP.

Rats were shipped from the U.S. Environmental Protection Agency research facility in Research Triangle Park, NC to the University of Illinois at Urbana-
Champaign for behavioral testing on PND 85 in Cohort 1 and on PND 115 in Cohort 2. Rats were given 2 weeks to acclimate, during which time food and tap water were available ad libitum. Pairs or triples of rats were housed in standard plastic cages containing corn-cob bedding in a temperature- and humidity-controlled room (22°C, 40–55% humidity), and animals were maintained on a 12-h reverse light-dark cycle (lights off at 0900). One week prior to RAM testing, feed was reduced to achieve approximately 85% of free-feeding body weight. Rats were food-restricted during behavioral testing for 14 weeks. During this time period, they were weighed 5 days/week, and their food intake was adjusted as needed to maintain a consistent body weight. All behavioral testing took place Mondays through Fridays during the dark phase of the light cycle. Both animal facilities are AAALAC-approved and all procedures were in accordance with protocols approved by the Institutional Animal Care and Use Committees of both institutions.

**Radial-arm maze (RAM).** Rats were tested on the RAM beginning at 120 to 150 days of age. Testers were trained by the same individual and periodically checked by that individual to insure accurate and standard testing procedures across testers. All testers remained blind to the treatment conditions of the rats throughout testing. The 12-arm radial maze, which has also been used in previous studies (i.e., See et al., in press), was made of gray poly-vinyl chloride and stood 84 cm above the floor. The maze consisted of a 34-cm circular platform with 12 arms (65 cm long × 9.5 cm wide with 2.5-cm walls) extending from it radially. Each arm was partially enclosed by a clear sloping barrier (18 cm high at the hub, descending to a height of 8 cm at the end) that extended 34 cm into the arm and prevented rats from entering adjacent arms without re-entering the central hub. Recessed food cups located at the ends of the arms were baited with a food reinforcer (1 Kellogg’s Froot Loop). Rats were tested in a dimly lit room with numerous extra-maze spatial cues.

Rats were “shaped” for 5 days during the week prior to testing. Shaping consisted of placing the rat in an opaque ring in the central hub with 4 food reinforcers for 5 min or until all reinforcers were eaten. Rats were tested using a working/reference memory paradigm (Honig, 1978; Olton et al., 1979) in which only 8 of the 12 arms were baited with a food reinforcer. Six distinct reinforcement patterns were selected with the restriction that there would be no more than 3 consecutively baited arms. The pattern of baited and unbaited arms remained consistent across test sessions for each animal, with the 6 patterns counterbalanced across sex and exposure group. For testing, the rat was placed inside an opaque ring on the center platform. After 10 s, the ring was lifted, and the rat was free to roam the maze. The session was terminated when the rat had retrieved all 8 baits or after 8 min had elapsed. The hub and arms of the maze were wiped with a water-dampened sponge between rats to diffuse odor cues. An arm entry was operationally defined as having all 4 paws inside an arm. The tester used a computer program developed within the lab to record the total session time and the sequence of arm entries and food reinforcers eaten. The number of working memory errors (re-entries into previously baited arms) and reference memory errors (entries into unbaited arms) were calculated by the computer program, along with average duration per arm (total session time divided by total number of arm entries). Rats were tested once/day, 5 days/week, for 12 weeks (60 sessions).

**Drug challenges.** Rats were challenged with dizocilpine (MK-801) (0.025, 0.05, 0.10 mg/kg, sc), scopolamine (0.02, 0.06, 0.18 mg/kg, sc), and a saline control during weeks 8 to 11. Drug challenges were given on Tuesdays and Fridays to allow at least 3 drug-free days preceding each drug challenge. The drugs were administered via subcutaneous injection 20 min prior to testing. Each rat received each of the 7 drug doses (3 doses each of dizocilpine and scopolamine plus 1 saline control) one time in a counterbalanced order. Drugs were purchased from Research Biochemicals International (Natick, MA). The high doses of both dizocilpine and scopolamine were set at a concentration that had shown marked memory impairments in previous studies (Levin et al., 1998; Levin and Torry, 1996), and lower doses were chosen to allow for the evaluation of subtle drug effects and a dose-response relationship.

**Data analysis.** The data were analyzed via repeated measures analysis of variance (ANOVA) using SPSS 7.5 for MS Windows. The litter was used as the unit of analysis nested within exposure group, and sex was treated as a nested within litter variable. Working and reference memory errors were averaged into weekly blocks of 5 sessions, except during drug challenges (weeks 8–11) when only the 3 non-drug days were used in the session-block average. The session-block means of working and reference memory errors were analyzed via separate 3-way ANOVAs with exposure group as a between-litter variable, sex as a nested-within-litter variable, and session-block as a nested-within-rat variable. For the drug challenges, the 3 doses of dizocilpine and the saline control were analyzed via separate 3-way ANOVAs for working and reference memory errors, with exposure group as a between-litter variable, sex as a nested-within-litter variable, and drug dose (0.025, 0.05, or 0.10 mg/kg, sc dizocilpine and saline control) as a nested-within-rat variable. Similarly, the 3 doses of scopolamine plus the saline control were analyzed via separate 3-way ANOVAs for working and reference memory errors, with exposure group as a between-litter variable, sex as a nested-within-litter variable, and drug dose (0.02, 0.06, or 0.18 mg/kg, sc scopolamine and saline control) as a nested-within-rat variable. For the drug challenges, data from rats that entered less than 6 baited arms were excluded from the analyses, because choice accuracy could not be properly evaluated given their general unresponsiveness on the maze following the injections. A total of 4 litters (2 control and 2 A1254) were excluded from the scopolamine analyses because either the male or the female from those litters had entered less than 6 baited arms on one or more of the scopolamine drug challenge days. No litters were removed from the dizocilpine drug challenge analyses for this reason. Significant effects were further analyzed via tests for simple main effects and/or planned comparisons of the A1254-exposed group to the control groups, as appropriate (Keppel, 1982). Statistical significance was ascribed at p < 0.05.

**RESULTS**

During testing, 2 A1254-exposed males died unexpectedly, one from a urinary tract obstruction, after 28 days of testing and the other after 48 days of testing, from an undetermined cause. It is unclear whether these deaths were related to the A1254 exposure. Data from these 2 litters were excluded from the analyses.

Control and A1254-exposed rats did not differ in the mean number of food reinforcers eaten during shaping (3.48 ± 0.12 and 3.21 ± 0.29 food reinforcers ± SEM for the control and 6-mg/kg A1254 groups, respectively), suggesting that the 2 groups were not differentially motivated at the beginning of testing. Both groups of rats learned the maze as illustrated in the learning curves in Figures 1 and 2. Highly significant main effects of session block for both working (F 11,176 = 55.135, p < 0.001) and reference memory (F 11,176 = 72.482, p < 0.001) errors further support this claim.

For working memory errors, the main effect of exposure group was not statistically significant: F 1,16 = 3.648, p = 0.074. Although the exposure-by-sex interaction did not reach statistical significance (F 1,16 = 2.917, p = 0.017), the exposed males appeared to be more impaired than the exposed females (Fig. 1). Therefore, the data for the 2 sexes were analyzed separately. The analysis of working memory errors separately for males revealed a highly significant main effect of exposure: F 1,16 = 19.183, p < 0.001 (Fig. 1A, inset), indicating that A1254-exposed males made significantly more working memory errors than control males (2.15 ± 0.13 and 3.20 ± 0.18 errors ± SEM for control and A1254 males, respectively).
When analyzed separately, A1254-exposed females did not make more working memory errors than control females, as revealed by a non-significant main effect of exposure (Fig. 1B, inset). Separate analyses by sex also revealed a significant block by exposure interaction for females (F $11,176 = 1.990$, $p = 0.032$). A1254-exposed females continued to improve during the drug challenges, while control female performance was disrupted and more variable during the drug challenges (Fig. 1B). Inspection of the data for individual animals indicated that this effect was primarily due to the aberrant performance of one control female.

For reference memory errors, there was a significant exposure-by-sex interaction, $F_{1,16} = 5.033$, $p = 0.039$. Analyses of each sex separately revealed a significant main effect of exposure in the males: $F_{1,16} = 5.033$, $p = 0.039$ (Fig. 2A, inset). A1254 males made significantly more reference memory errors than control males (3.17 ± 0.10 and 4.13 ± 0.14 errors ± SEM for control and A1254 males, respectively). In contrast, the mean number of reference memory errors did not significantly differ between A1254 and control females (Fig. 2B, inset).

There was a significant main effect of sex on average duration per arm (seconds/arm entry): $F_{1,16} = 5.426$, $p = 0.033$. Females had significantly shorter latencies (16.10 ± 0.13 s/arm entry) than males (17.68 ± 0.15 s/arm entry). A highly significant main effect of session-block for average duration per arm was also discovered, $F_{11,176} = 12.288$, $p < 0.001$. Scheffe mean contrasts revealed a significant decrease in average duration per arm from the first session block to the second, and an increase in average duration per arm for the non-drug challenge days of session-block 10 compared to session-blocks 2–5.

The dizocilpine and scopolamine drug challenges did not differentially affect RAM performance in control and A1254-exposed animals. For dizocilpine, the main effect of A1254 exposure was not significant for working (Fig. 3A) or reference memory errors (Fig. 3B). The main effect of sex and the A1254 by sex interaction were not significant for either working or reference memory errors. Thus, Figure 3 compares control and A1254-exposed animals collapsed across sex. The A1254 exposure by dizocilpine dose interaction for working or reference memory errors was also not significant. Highly significant main effects of the dizocilpine dose (saline control, 0.025, 0.05, or 0.10 mg/kg) were observed for both working ($F_{3,48} = 38.441$, $p < 0.001$) and reference memory errors.
over the 0.02-mg/kg dose (Fig. 4A).

Scopolamine doses of 0.02 and 0.06 mg/kg were also significantly increased over the saline control and the 2 lower doses (0.02 and 0.06 mg/kg; Fig. 3). For working memory errors, there were also significantly higher than at the 0.25 and 0.10 mg/kg doses (Fig. 3). For working memory errors, there was a significant sex by dizocilpine dose interaction ($F_{3,48} = 2.15$, $p < 0.05$). Paired comparisons of the 2 sexes at each dose revealed no significant differences, but at the highest dose (0.10 mg/kg), there was a slight trend for females to make more working memory errors than males (18.28 ± 2.89 vs. 12.06 ± 2.15 errors ± SEM for females and males, respectively; $t(17) = 1.689, p = 0.110$).

For scopolamine, the main effect of A1254 exposure was not significant for working (Fig. 4A), or reference memory errors (Fig. 4B). Again, the main effect of sex and the A1254 by sex interaction were not significant for either working or reference memory errors, so Figure 4 illustrates mean performance of control and A1254-exposed rats combined across sex. Also, the A1254 exposure by scopolamine dose interaction was not significant for either working or reference memory errors. Highly significant main effects of scopolamine dose (saline control, 0.02, 0.06, 0.18 mg/kg) were observed for both working and reference memory errors, $F_{3,36} = 57.198, p < 0.001$, and reference memory errors, $F_{3,36} = 14.657, p < 0.001$. Scheffe mean contrasts revealed that only the highest dose of scopolamine (0.18 mg/kg) were significantly increased over the saline control and the 2 lower doses (0.02 and 0.06 mg/kg; Fig. 4). Mean working memory errors following the 0.06 mg/kg dose of scopolamine were also significantly increased over the 0.02-mg/kg dose (Fig. 4A).

**DISCUSSION**

**Gestational-Lactational A1254 Exposure Impairs RAM Performance in Males**

Gestational and lactational exposure to A1254 resulted in significantly more working and reference memory errors in male rats. A1254-exposed females were not statistically different from control females. The increase in errors in A1254 males relative to control males began in the first block of testing and was evident in nearly every testing block. The persistence of the effect indicates that it was not merely an acquisition deficit. Drug challenges with dizocilpine or scopolamine did not differentially affect maze performance of control and exposed animals.

The results from this study are in agreement with previous studies that have found learning and memory deficits in monkeys and rats exposed to PCBs during prenatal and/or early postnatal development. Several non-human-primate studies have found impairments on delayed spatial alternation (DSA) and spatial discrimination reversal learning, as well as on fixed interval and differential reinforcement of low-rate operant schedules, following developmental exposure to PCBs (Bowman et al., 1978; Levin et al., 1988; Rice, 1997, 1998; Rice and Hayward, 1997; Schantz et al., 1991). Rodent studies have documented impairments on DSA (Schantz et al., 1995), retention of visual discrimination (Lilienthal and Winneke, 1991), water-filled multiple T-maze learning (Shiota, 1976), and active avoidance learning (Lilienthal and Winneke, 1991; Panteleoni et al., 1988; Storm et al., 1981; Tilson et al., 1979). However, not all studies in rodents have found deficits following developmental PCB exposure. For instance, individual coplanar PCB congeners do not appear to produce learning deficits. Studies in Long-Evans rats exposed to coplanar PCB 126 throughout gestation and lactation failed to find any deficits on a number of learning and attention measurements, including DSA, sustained attention, visuospatial attention, and several different operant schedules of reinforcement (Bushnell and Rice, 1999; Rice, 1999; Rice and Hayward, 1998, 1999).

In a separate study, the offspring of Sprague-Dawley rats dosed from GD 10 to GD 16 with coplanar PCB congeners (PCB 77 or 126) were not impaired on DSA or on the 8-arm RAM (Schantz et al., 1996). Schantz et al. (1995) also did not find impairments on the 8-arm RAM in male or female offspring of Sprague-Dawley rats exposed to individual ortho-substituted PCB congeners (PCB 28, 118, and 153) on GD 10–16, although the female rats in that study were impaired on a DSA task.

When comparing the current findings to the previous RAM studies, 3 points must be considered. First, the previous studies (Schantz et al., 1995, 1996) were done using an 8-arm maze. The 12-arm working-reference memory task used in the current study may be more difficult than the tasks previously employed.
on the 8-arm maze. As such, it may be able to detect more subtle impairments in cognitive function. Second, the lack of effects found in previous RAM studies was following exposure to selected individual PCB congeners. The impairment in the current study was following exposure to a complex commercial PCB mixture that contains a large number of congeners with diverse chemical structures and toxicity profiles, as well as dibenzofuran contaminants. The congeners in the A1254 mixture could interact in complex ways to produce learning deficits. Third, the previous 8-arm RAM studies also involved a shorter period of maternal exposure (GD 10–16 vs. GD 6 to PND 21 used in this study). It is possible that the previous studies did not expose the offspring to sufficient PCBs or did not expose during the appropriate critical period to result in RAM deficits.

In contrast to our RAM findings, Gilbert and colleagues did not observe any deficits in performance on the Morris water maze (MWM) in A1254-exposed littermates of the animals we tested (see companion study by Gilbert et al., 2000). Although both the RAM and the MWM are hippocampally-mediated spatial learning tasks (Morris et al., 1986; Olton et al., 1979), it is debated that the 2 tasks are fundamentally different, varying in task motivation, stress, and speed of task acquisition. Hodges (1996) reports that behaviors in the RAM and MWM are not necessarily closely correlated and that these “similar” maze tasks may actually be examining quite different processes. These differences might explain the lack of spatial learning deficits on the MWM following gestational-lactational PCB exposure.

**A1254-Exposed Rats Are Not More Impaired by Dizocilpine and Scopolamine Challenges**

Drug challenges with the NMDA antagonist dizocilpine or muscarinic receptor antagonist scopolamine did not differentially affect maze performance of control and exposed animals. The drug challenges were planned with the hypothesis that they might “unmask” or further exacerbate PCB-related effects. However, given the frank learning and memory deficits observed in the male rats, it would be interesting to investigate whether administering NMDA or muscarinic ACh agonists would ameliorate the observed deficits. Further, administering the drug challenges during acquisition rather than once the task was well learned might yield different results.

Although exposed and control animals did not perform differently during the drug challenges, it does appear that the highest drug doses effectively impaired memory. Increases in both working and reference memory errors were observed in all groups with the highest doses of dizocilpine and scopolamine. However, it should be noted that the dose selection for dizocilpine was not optimal. At the high dose, 0.10 mg/kg, some motor impairment was observed, confounding the mnemonic effects. In addition, we failed to observe a dose-response relationship since the intermediate dose of 0.05 mg/kg did not yield an intermediate increase in errors. The impairments in both working and reference memory following the 0.10 mg/kg dose of dizocilpine do, however, replicate previous findings (e.g. Levin et al., 1998).

In contrast, the scopolamine doses gave a suggestion of a dose-response curve with increasing doses resulting in increasing working and reference memory errors. Controversy exists as to whether the cholinergic system in the brain is involved in working memory selectively, or if it is involved in both working and reference memory. Using the working/reference memory paradigm on the RAM, some investigators report that scopolamine selectively impairs working memory (Beatty and Bierley, 1985; Fader et al., 1999; Levin et al., 1997; Wirsching et al., 1984), whereas other experiments, including ours, have found impairments in both working and reference memory errors (Beninger et al., 1995; Buhot et al., 1995; Miyagawa et al., 1995; Okaichi and Jarrod, 1982; Okaichi et al., 1989; Wang and Tang, 1998; Xiong and Tang, 1995). In conclusion, the lack of differential amnestic effects of scopolamine and dizocilpine in the control and PCB-exposed groups provides preliminary evidence in support of the hypothesis that selective alterations in the muscarinic ACh or NMDA glutamate systems are not mediating the observed learning deficits.

**Sex-Specific Deficits following Gestation-Lactation Exposure to A1254**

The working and reference memory deficits in the current study were confined to the A1254-exposed male rats, whereas the exposed female rats performed similarly to control females. A male-specific effect was not anticipated, particularly since previous work in our lab found that impairments on a T-maze DSA task were present only in the PCB-exposed females and not in exposed males (Schantz et al., 1995). As discussed above, the animals in that study were exposed to individual ortho-substituted PCB congeners (PCB 28, 118, or 153) from GD 10–16, while the animals in the current study were exposed to a complex commercial PCB mixture from GD 6 to weaning. Certain effects of PCBs could be either masked or unmasked when specific congeners from the mixture are given individually. The different exposure paradigms could also affect the occurrence or degree of resulting impairments.

The mechanism for the sex-specific effects of PCBs on the RAM and other learning tasks is unknown, but it can be speculated that endocrine disruption may be responsible. PCBs have complex effects on multiple endocrine systems. PCBs have been shown to reduce circulating thyroxine concentrations (Brouwer et al., 1998) and also have a complex array of estrogenic and anti-estrogenic effects (Hansen, 1998; Hany et al., 1999). Alterations in either the thyroid or gonadal hormone systems during the period of brain development could affect learning and memory in male and female rats differentially (Davenport, 1976; van Haaren et al., 1990; Williams et al., 1990).
Male rodents typically make fewer errors than females on spatial learning tasks (Williams and Meck, 1991), and this difference is modulated by the organizational actions of estrogen in the developing male brain (MacLusky and Naftolin, 1981). While the overall numbers of working and reference memory errors for the male and female controls in the present experiment did not differ significantly, the sex-by-session-block interaction terms were significant for both working and reference memory errors. Females made more errors than males primarily during the later blocks of testing.

Alterations in thyroid hormones during early development can alter sexual differentiation of the brain indirectly by shortening (hyperthyroidism) or lengthening (hypothyroidism) the critical period for estrogen exposure (MacLusky et al., 1998). Thyroid hormone can also inhibit estrogen’s actions directly at the genomic level (Zhu et al., 1996). Thus, agents that increase thyroid hormone bioavailability, or mimic the actions of thyroid hormone would be expected to attenuate estrogen-mediated responses, whereas agents that reduce or block thyroid hormone action would have the opposite effect.

One intriguing explanation for the pattern of effects seen in the current study would be that spatial learning in the males has been demasculinized. There is recent evidence that rats born to dams dosed with 4 mg/kg/day of A1254 have significantly reduced tests weights and serum testosterone levels in adulthood (Hany et al., 1999). Testosterone was not measured in the neonatal period, so it is unknown whether testosterone was also decreased during the critical period for sexual differentiation of the brain.

Alternatively, demasculinization could be mediated by the actions of A1254 on the thyroid system. A1254 dramatically decreases T4 and slightly reduces triiodothyronine (T3) levels (Goldey et al., 1995), yet the developmental effects of A1254 exposure are not fully consistent with hypothyroidism. Zoeller et al. (2000) recently reported that A1254 causes thyroid hormone-like elevations in the expression of RC3/neurogranin and myelin basic protein, 2 key thyroid hormone responsive genes in the developing brain. Chemical goitrogens, such as propylthiouracil and methimazole, reduce the expression of these same genes (Ibarrola and Rodriguez-Pena, 1997; Iniguez et al., 1996). These new findings suggest that A1254 may act as a thyroid hormone mimic in the brain. Cheek et al. (1999) investigated the affinity of hydroxylated PCBs for recombinant human thyroid receptor β in vitro and found it to be 10,000-fold lower than the affinity of T3 for the receptor, suggesting that PCBs are not acting through binding to the β receptor. However, the affinity of PCBs for the thyroid receptor α has not been investigated. Furthermore, it is possible that A1254 may be acting as a thyroid hormone mimic via an indirect mechanism, such as enhancing thyroid hormone uptake into tissues or increasing the conversion of T4 to T3. If A1254 is acting as a thyroid mimic in the brain, this could result in an attenuation of estrogen’s actions and lead to incomplete sexual differentiation and poorer spatial learning in developmentally exposed male rats (MacLusky et al., 1998).

**LTP as a Potential Underlying Neurophysiological Mechanism**

Gestational and lactational exposure to A1254 reduces the magnitude of LTP in the dentate gyrus of adult male offspring (Gilbert and Crofton, 1999). PCB-induced increases in the threshold for LTP induction were also observed in male littermates of some of the animals tested in the present study (Gilbert et al., submitted). Findings of both learning deficits on the RAM and reduced hippocampal LTP in male rats gestationally and lactationally exposed to A1254 suggest a causal relationship. Other authors have implicated a relationship between RAM and LTP (e.g., Balschun et al., 1999; Butelman, 1990; Ishihara et al., 1997). Interestingly, Pavlides et al. (1991) found a significant correlation between reductions in dentate gyrus LTP and spatial learning deficits on an 8-arm RAM following neonatal hyperthyroidism. Rats treated with T3 neonatally made more RAM errors and the efficacy of LTP induction was attenuated (Pavlides et al., 1991). This study also found sex-specific differences in the effects of neonatal T3 administration on the induction of LTP, suggesting that the neonatal hyperthyroidism may have affected LTP in females to a lesser extent than males. LTP has not been evaluated in A1254-exposed females, so at present we cannot determine if a sex-specific impairment is also present following A1254 exposure.

However, if A1254 is acting as a thyroid hormone agonist in the brain (Zoeller et al., 2000), these observations provide a potential explanation for the sex-specific RAM learning deficit we observed in A1254-exposed male rats. They also support the hypothesis that reduced capacity for synaptic plasticity in the hippocampus, as evidenced by alterations in LTP induction, may be the underlying neurophysiological mechanism for the observed cognitive deficits following gestational-lactational PCB exposure.

**Summary and Conclusions**

Deficits in both working and reference memory were observed in male offspring following maternal A1254 exposure during gestation and lactation. Drug challenges with the NMDA antagonist dizocilpine and the muscarinic antagonist scopolamine did not differentially affect working or reference memory of control and exposed rats. A number of conclusions can be drawn from these findings. First, gestational-lactational exposure to a complex PCB mixture caused lasting deficits in spatial learning and memory in male rats. Sex-specific learning and memory effects following gestational-lactational PCB exposure need to be further investigated, given this male-specific deficit on the RAM and a previous female-specific deficit on a T-maze DSA (Schantz et al., 1995). Second, neither antagonizing the cholinergic nor the glutamatergic neurotransmitter
systems exacerbated the adverse effects of gestational-lactational PCB exposure. Lastly, alterations in LTP induction may underlie the observed cognitive deficits following gestational-lactational PCB exposure.

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