Spatial Alternation Deficits Following Developmental Exposure to Aroclor 1254 and/or Methylmercury in Rats

John J. Widholm,*† Sherilyn Villareal,‡ Richard F. Seegal,§ and Susan L. Schantz†§

*Department of Psychology, College of Charleston, Charleston, South Carolina 29424; †Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61802; and ‡Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201, and School of Public Health, University at Albany, State University of New York, Albany, New York 12222; and §Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Received July 26, 2004; accepted September 16, 2004

Key Words: PCBs; MeHg; methylmercury; delayed spatial alternation; DSA; rats.

Polychlorinated biphenyls (PCBs) and methylmercury (MeHg) are ubiquitous environmental contaminants that have established neurotoxic effects. Both compounds are resistant to biodegradation and accumulate in the aquatic food chain of both marine and freshwater environments. Contaminated fish and seafood represent the primary source of PCB and MeHg exposure in humans and wildlife. PCBs and MeHg pose special problems for the developing child because they are transferred from mother to fetus via the placenta (Amin-Zaki et al., 1981; Jacobson et al., 1984; Kajiwara et al., 1996; Kodama and Ota, 1977) and from mother to infant through lactation (Masuda et al., 1978; Sakamoto et al., 2002; Yakushiji et al., 1984) and thus are present during critical periods of development. Because co-exposure to PCBs and MeHg is possible, an understanding of the possible effects of combined developmental exposure to these contaminants is necessary.

The impact of developmental exposure to either PCBs or MeHg on cognitive function in humans has been assessed in several epidemiological studies. Jacobson and colleagues have reported PCB-related cognitive deficits in a cohort of Michigan children including impairments in visual recognition memory during infancy (Jacobson et al., 1985), poorer performance on verbal and memory tests at 4 years of age (Jacobson et al., 1990), and lower IQ, reduced attention, and impaired response inhibition at 11 years of age (Jacobson and Jacobson, 1996, 2003). Similar effects were observed in a cohort of children from the Oswego, NY area in which PCB exposure due to consumption of contaminated fish from Lake Ontario was associated with reductions in visual recognition memory at 6 and 12 months of age (Darvill et al., 2000) and poorer cognitive function at 3.5 years of age (Stewart et al., 2003b). Unlike the findings of the Michigan cohort, when these same children were tested at 4.5 years of age PCB exposure was no longer associated with reduced performance on the cognitive tests, but response inhibition, an aspect of executive function, was impaired on an attentional task in the

Polychlorinated biphenyls (PCBs) and methylmercury (MeHg) are ubiquitous environmental contaminants that alter cognitive function in both humans and animals. Because PCBs and MeHg often occur together in the environment, it is important to understand whether these two contaminants have the potential to interact, causing additive or greater than additive effects. The current study examined the combined effects of gestational and lactational exposure to Aroclor 1254 (A1254), a commercial PCB mixture, and MeHg on a series of spatial alternation tasks including cued spatial alternation (CA), non-cued spatial alternation (NCA), and delayed spatial alternation (DSA) in rats using standard two-lever operant testing chambers. Pregnant Long-Evans rats received either 6 mg/kg A1254 pipetted onto a Keebler Vanilla Wafer cookie (PCB-only group), 0.5 ppm. MeHg dissolved in the drinking water (MeHg-only group), 6 mg/kg A1254 + 0.5 ppm. MeHg (PCB + MeHg group), or corn oil vehicle and normal tap water (control group) beginning 28 days prior to mating and continuing through postnatal day 16. One male and one female from each litter began testing on spatial alternation at approximately 110 days of age. Animals were reinforced for pressing the lever opposite that pressed on the previous trial. In general, animals exposed to A1254 and/or MeHg were impaired relative to control rats on the NCA and DSA tasks. Significant reductions in NCA performance were observed in the MeHg-only and PCB + MeHg groups, while significant reductions in DSA performance were observed in the PCB-only and MeHg-only groups. The PCB + MeHg group showed a similar magnitude reduction in performance on DSA, but this difference was not statistically significant due to increased variability in that group. The reductions in DSA performance were observed across most of the delays, indicating that memory impairments were not likely the cause of the deficit. Instead, the DSA deficits following exposure to A1254 and/or MeHg are indicative of either an associative or attentional impairment. The results from the current study indicate that combined exposure to PCBs and MeHg does not exacerbate the PCB- or MeHg-induced impairments on spatial alternation tasks.
more highly PCB-exposed children (Stewart et al., 2003a,b). In a cohort of Dutch children PCB exposure was associated with lower scores on both sequential and simultaneous processing tasks at 3.5 years of age (Patandin et al., 1999). At 6.5 years of age PCB exposure was no longer associated with cognitive impairment (Vreugdenhil et al., 2002). However, similar to the Michigan and Oswego cohort, when a subset of the Dutch cohort was tested again at 9 years of age, impairments in attention and executive function were observed in these children (Vreugdenhil et al., 2004). Lastly, findings from a German cohort of children have revealed cognitive impairments associated with PCB exposure at 2.5 and 3.5 years of age, but not earlier (Walkowiak et al., 2001; Winneke et al., 1998). The cognitive impairments seen in the more recent Oswego and Dutch cohorts occurred at lower PCB levels than were observed in the Michigan cohort, while the PCB levels of the German cohort were slightly higher (Longnecker et al., 2003).

Not all studies have reported PCB-related cognitive deficits. In a cohort of North Carolina children, no effects of PCBs on cognitive function were observed between 3 and 5 years of age (Gladen and Rogan, 1991), even though PCB exposure in these children was significant enough to be associated with delayed psychomotor development through 2 years of age (Gladen et al., 1988; Rogan et al., 1986; Rogan and Gladen, 1991).

Longitudinal studies assessing the effects of environmental MeHg exposure on cognitive functioning in children have also been conducted. In the Faroe Islands, prenatal MeHg exposure from maternal consumption of MeHg-contaminated pilot whale meat was associated with deficits in memory, language, and attention at 7 years of age (Grandjean et al., 1997, 1998). PCBs, which are also found in pilot whales, had little effect on cognitive function except in the highest MeHg-exposed individuals (Grandjean et al., 2001). Similarly, prenatal exposure to MeHg in a cohort of New Zealand children was associated with language, perceptual, and full-scale IQ deficits at 6 years of age (Crump et al., 1998; N.A.S., 2000). In contrast, no cognitive deficits were found in a cohort of Seychellois children prenatally exposed to dietary MeHg from contaminated ocean fish when tested at 5.5 years of age (Davidson et al., 1998).

Animal studies of cognition following developmental PCB and/or MeHg exposure, in general, support the findings of the human epidemiology studies. Schantz and colleagues found that exposure of monkeys to commercial PCB mixtures during gestation and lactation resulted in impairments on several spatial learning and memory tasks including an increase in trials to criterion on the early reversals of a spatial discrimination-reversal learning (spatial RL) task (Bowman et al., 1978; Schantz et al., 1989) and decreased accuracy on a delayed spatial alternation (DSA) task (Levin et al., 1988). Low-level postnatal-only exposure of monkeys to a PCB congener mixture that was representative of the PCBs found in human breast milk was also found to significantly impair the learning of DSA (Rice, 1999a; Rice and Hayward, 1997) and disrupt differential reinforcement of low rates (DRL) schedule performance (Rice, 1998). Unlike the Schantz et al. (1989) study, no impairments in spatial RL were observed in the postnatally PCB-exposed monkeys (Rice, 1998, 1999a).

Deficits in spatial RL, DSA, and radial arm maze (RAM) learning have also been documented in rats following pre- and/or postnatal exposure to commercial PCB mixtures (Roegge et al., 2000; Widholm et al., 2001) or individual ortho-substituted PCB congeners (Schantz et al., 1995). Widholm et al. (2001) demonstrated a sex-specific impairment on spatial RL following developmental exposure to A1254, with males exhibiting impairments on the first reversal and females on later reversals. Sex-specific effects have also been demonstrated on the 12-arm RAM following A1254 exposure, with males exhibiting impairments while females were unaffected (Roegge et al., 2000), and on DSA with developmental exposure to only ortho-substituted PCBs (PCB 28, 118, 153) causing deficits in the PCB-exposed females, but not the PCB-exposed males. However, the ability of PCBs to consistently alter cognitive function in rats becomes less clear when one considers the recent studies by Zahalka et al. (2001) and Bushnell et al. (2002) in which perinatal exposure to commercial PCB mixtures at doses similar to the studies of Widholm et al. (2001) and Roegge et al. (2000) failed to cause clear impairments on DSA or Morris water maze performance (Zahalka et al., 2001) and sustained attention (Bushnell et al., 2002). Similarly, exposure to coplanar PCB congeners has not resulted in deficits on a variety of cognitive tasks including DSA (Rice, 1999b; Schantz et al., 1996), visual-spatial attention (Bushnell and Rice, 1999), or radial arm maze learning (Schantz et al., 1996).

The effects of perinatal MeHg exposure on cognitive function in animals have also been mixed. In a series of experiments in monkeys perinatally exposed to MeHg, visual discrimination/reversal learning was slightly facilitated when compared to controls, while performance on a fixed interval task indicated deficits in temporal discrimination, with exposed monkeys responding earlier in the interval than controls (Rice, 1992). In a separate series of experiments, MeHg-exposed infant monkeys showed memory deficits on both an object permanence task (Burbacher et al., 1986) and a visual recognition memory task (Gunderson et al., 1988). However, later in life these same animals were not impaired on another memory-dependent task, DSA (Gilbert et al., 1993) and actually showed a slight, but significant improvement in performance. MeHg-exposed rats exhibited poorer performance on a schedule in which reinforcement was contingent on emitting a specified number of responses within a limited time period (differential reinforcement of high rates, or DRH schedule). As the DRH response requirement became more difficult, the MeHg-exposed rats earned fewer reinforcements (Bornhausen et al., 1980; Newland and Rasmussen, 2000). These results are suggestive of a reduced sensitivity to the changes in reinforcement contingencies as the response requirement became greater, although effects on motor function cannot be ruled out.
Because the predominant exposure model in the animal literature has been to single contaminants, the potential for additive or interactive neurotoxic effects from combined exposure to PCBs and MeHg is unknown. However, support for the hypothesis that PCBs and MeHg have the ability to interact has been provided by recent in vitro work on dopamine and calcium concentrations in nerve cells (Bemis and Seegal, 1999, 2000). Dopamine is an important neurotransmitter for many cognitive processes including memory and attention (e.g., Brozoski et al., 1979). Bemis and Seegal (1999) found that in vitro exposure of rat brain striatal punches to MeHg and PCBs resulted in markedly greater reductions in dopamine levels than when the exposure was to PCBs or MeHg alone, suggesting a synergistic interaction between these two compounds (Bemis and Seegal, 1999). Furthermore, Bemis and Seegal (2000) demonstrated the potential for synergistic and/or antagonistic interactions on intracellular calcium concentrations in rat cerebellar granule cells following coexposure to PCBs and MeHg. If PCBs and MeHg are able to act similarly in vivo, coexposure to PCBs and MeHg could place the organism at greater risk for cognitive impairments.

The goal of the current series of experiments was to examine whether exposure to a mixture of PCBs and MeHg in gestationally and lactationally exposed Long Evans rats would exacerbate the effects of spatial alternation (SA) tasks seen following exposure to PCBs alone. SA was chosen for study because it assesses both learning and memory within a single task, it has been shown previously to be sensitive to disruption by developmental PCB exposure, and because accurate performance on SA tasks is dependent on dopamine (Brozoski et al., 1979), which has been shown to be synergistically reduced in vitro following combined exposure to these contaminants (Bemis and Seegal, 1999). Therefore, the SA test battery should be a sensitive behavioral assay to elucidate the potential of PCBs and MeHg to interact to produce effects on cognitive function.

MATERIALS AND METHODS

Animals, exposure, and mating. Sixty-three primiparous female Long-Evans rats (Harlan, Madison, WI), approximately 60 days old, were shipped to the University of Illinois for dosing and mating. The animals were shipped in three cohorts of approximately 20 females each, spaced 6 months apart. The females were individually housed in standard plexiglass rat cages with ground corncob bedding in an environmentally controlled room (22°C, 40–55% humidity) on a 12-hour reverse light-dark cycle (lights off at 0900). The corncob bedding was independently analyzed for a variety of contaminants by the Illinois Department of Agriculture including PCBs and mercury concentrations (detection limit = 0.1 ppm and 0.02 ppm for PCB and mercury analyses, respectively) and found to have nondetectable levels of all contaminants except arsenic (0.03 ppm; detection limit = 0.02 ppm). The females were weighed daily and were assigned to the PCB, MeHg, PCB + MeHg, or control group by counterbalancing for body weight. For each cohort, there were at least five females assigned to each of the four exposure groups with any additional rats assigned to groups that had fewer litters due to reduced mating success in previous cohorts. Beginning one week after arrival, the females were weighed and dosed daily at approximately 11:00 a.m. for 66 consecutive days. PCB-only exposed females were fed one-half of a Keebler Vanilla Wafer cookie onto which 6 mg/kg Aroclor 1254 (A1254; Lot #124–191; AccuStandard, New Haven, CT) dissolved in corn oil vehicle was pipetted at a volume of 0.4 ml/kg. This dose of A1254 was selected because it does not result in increased pup mortality and causes only a moderate amount of postnatal weight loss (e.g., Crofton et al., 2000). MeHg-only exposed females received drinking water in which methylmercuric chloride (Alfa Aesar Chemicals, Ward Hill, MA) was dissolved at a concentration of 0.5 μg/ml (0.5 ppm). The MeHg-contaminated drinking water was available continuously in the home cage, and the water bottles were weighed daily to monitor water intake.

PCB + MeHg-exposed females were fed the 6-mg/kg A1254-contaminated cookies, and the 0.5-ppm MeHg-adulterated drinking water was available ad libitum. Control females were fed vanilla wafer cookies that contained only the corn oil vehicle and received unadulterated tap water.

Mating began 28 days after the beginning of dosing in which each female was paired with an unexposed male Long Evans rat. The same male–female pairs were housed together daily until conception occurred or eight days had elapsed at which point mating ceased. Conception was determined by the presence of a sperm plug and defined as gestational day 0 (GD 0). Females that did not give birth were kept for 21 days after the last day of mating and their uteri were dissected and examined for the existence of implantation sites.

On the day of parturition, the pups were examined for gross abnormalities, sexed, weighed, and the number of stillborn pups noted. Throughout the postnatal period, the pups were weighed periodically to monitor them for signs of overt toxicity. On PND 2, the litters were culled to 10 pups and balanced for gender whenever possible. On PND 16, all dosing ceased, and the pups were weaned from the dam on PND 21. At weaning, one male and one female pup from each litter were selected randomly for behavioral testing. The pups selected for behavioral testing were housed in same-sex, same-treatment pairs and were given ad lib access to food and water. They were weighed weekly until 70 days of age, at which point their food access was restricted to reduce their body weights to 85% of their free-feeding weight. The veterinary staff routinely examined the rats to ensure their health. Behavioral testing (i.e., autoshaping) began at 85 days of age and SA testing began at approximately 110 days of age. Testing occurred once/day, Monday–Saturday, during the dark phase of the light cycle. Each animal was weighed prior to each testing session and was supplementedly fed in its home cage at least 30 minutes after the session terminated to minimize the possible influence of noncontingent food on performance during the test sessions (e.g., Timberlake, 1984). The amount of feeding was adjusted daily by accounting for the amount of food earned in the daily test session and supplementing the rats with enough additional food to ensure the maintenance of desired body weights. All procedures were performed in AAALAC approved facilities and were in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Apparatus. Behavioral testing was conducted in 16 automated operant chambers (Med-Associates Inc., St. Albans, VT) housed in sound-attenuated wooden boxes, each ventilated by a fan (see Widholm et al., 2001). All operant chambers contained two retractable response levers and two stimulus cue lamps located symmetrically on both sides of the pellet trough. A white-noise generator masked extraneous sounds, and a sonalert speaker was used to signal reinforcement. The experimental contingencies were programmed using Med-State behavioral programming language (Med-Associates, St. Albans, VT).

Procedure All animals were shaped to press the response levers by using an autoshaping program and lever press training program that have been described in detail previously (see Newland et al., 1986; Widholm et al., 2001, 2003). Therefore, only a brief summary of each is included here.

Response shaping. At the beginning of the session, both response levers were extended into the chamber. Throughout this and all subsequent testing conditions, the white noise generator operated continuously during the test session to mask extraneous noises. The illumination of the cue-light above the right response lever was programmed according to a fixed-time 3-min schedule (FT-3 min) whereby the cue-light would be illuminated for 15 s duration every 3 min. Upon extinguishing of the cue-light, reinforcement was provided. If a lever
press occurred on either lever when the cue-light was illuminated, reinforcement was provided and the cue-light was immediately extinguished. Similarly, lever presses to either lever that occurred when the cue-light was not illuminated were reinforced. Reinforcement consisted of a single 45-mg food pellet (Formula A-1; Research Diets, New Brunswick, NJ) and the presentation of a 40-ms tone. Previous experience with this training procedure (e.g., Widholm et al., 2001) has shown that some rats respond to the lever associated with the illuminated cue-light and others respond to the lever associated with the darkened cue-light, thus the present methodology allows for either response to be reinforced. The FT-3 minute cue-light illumination schedule remained in effect until a total of 10 lever presses occurred on either response lever. Sessions terminated after 60 min had elapsed or 100 reinforcers were delivered, whichever occurred first. Criterion for this condition was set at 100 lever presses within a single session. All rats reached criterion in two to three days and there were no differences in number of days to criterion between exposure groups.

Lever-press training. Following autoshaping, all animals were exposed to a continuous reinforcement schedule (see Widholm et al., 2001, 2003) in which the cue-light and lever that was reinforced were alternated following the delivery of every fifth reinforcer. The purpose of this schedule was to strengthen the recently acquired lever press response and to prevent the rats from developing a lever or side preference prior to the start of cognitive testing. Single presses to the available lever resulted in reinforcement. After the receipt of the fifth consecutive reinforcer, the response lever was retracted, and the previously unavailable lever was then extended into the chamber, and the cue-light above the lever was illuminated. This cycle of lever alternation and cue-light illumination continued throughout the remainder of the session, terminating after either 100 reinforcers or 60 minutes had elapsed. A performance criterion of 100 reinforcers for at least two consecutive sessions was established for this condition. All rats completed the lever-press training in two or three sessions. There were no treatment-related effects on this task.

Spatial Alternation (SA).

1. Cued alternation training (CA). Prior to testing on CA, all rats were tested on a spatial reversal-learning task (spatial RL; see Widholm et al., 2001, 2003) in which the rats were reinforced for pressing the lever associated with a particular spatial location (either left or right) to a performance criterion of 85% correct for two consecutive sessions. Upon reaching criterion, the reinforced lever was reversed. Five reversals in addition to original learning (for a total of six phases) were conducted that lasted approximately 20 days. No significant differences were observed between control and treated groups on spatial RL (data not shown). Immediately following completion of the spatial RL task, the rats were trained on a CA task. For all of the alternation tasks (cued alternation, non-cued alternation, and delayed spatial alternation) the rats were reinforced for pressing the lever opposite the one pressed on the previous trial, regardless of whether that trial was correct or incorrect. Thus, if a rat pressed the right lever on a given trial (regardless of accuracy), it was then required to press the left lever on the following trial to receive a reinforcer. To facilitate acquisition of the alternation response on CA, trials were “cued” by illuminating the cue-light over the correct lever on each trial. There was no time limit for the rat to press a lever. The cue-light remained illuminated above the correct lever until a lever press occurred. For the first trial of the session, both cue-lights were illuminated and presses to either lever resulted in reinforcement. Thereafter, the rat was required to alternate its lever presses. A single press to the correct lever resulted in reinforcement, the retraction of the levers, and the extinguishing of the cue-light. A single press to the incorrect lever resulted in the retraction of the levers and the extinguishing of the cue-light. There was no delay imposed between trials, so upon retraction the levers were immediately extended back into the chambers and the cue-light above the correct lever was illuminated signaling the beginning of the next trial. Each session terminated after 200 trials had been presented or 90 min had elapsed, whichever occurred first. A performance criterion of 60% correct, a performance level just above chance, was established for this task.

2. Non-cued alternation training (NCA). Upon completion of the CA task, the rats were trained on the NCA task. NCA was identical to CA except that no cue-lights were used to signal the correct lever. Each rat was tested on NCA for 10 consecutive sessions, regardless of performance.

3. Delayed spatial alternation (DSA). Immediately following NCA testing, rats were tested on a DSA task. This task was identical to NCA except that variable delays of 0, 3, 6, 9, or 18 sec were imposed between trials. The delays were imposed randomly across the test session with the stipulation that the number of trials at each delay was balanced within each session and that a particular delay was not presented on more than three consecutive trials. Each rat was tested for 25 sessions regardless of performance.

Data analysis. For CA, cumulative errors to criterion served as the overall measure of learning. The number of errors was calculated by summing the total number of errors across all of the sessions in CA. Overall proportion correct served as the primary measure of learning for both NCA and DSA. For NCA, proportion correct was analyzed across all 10-test sessions to assess the rate at which learning took place, while proportion correct for DSA was analyzed by first transforming the 25 test sessions into 5-session block averages prior to analysis. For DSA, proportion correct at each delay was also examined to assess how performance changed as a function of delay. One concern when using an appetitive task to assess cognitive function is that performance may decrease toward the end of the session as the subjects become more sated. Therefore, the total number of errors by session quartile was examined for DSA to test for late-session reductions in accuracy. Lastly, average lever press latencies for correct and incorrect responses were analyzed for CA, NCA, and DSA tasks.

Response Pattern Analyses

In addition to these typical measures of overall performance, several response pattern analyses were conducted in order to better understand the potential cognitive changes produced by PCBs and/or MeHg. Specifically, these analyses were designed to assess whether exposed animals were more or less likely to exhibit a tendency to incorrectly respond following a correct or incorrect response (e.g., “win-stay” or “lose-stay” type errors). These analyses were conducted by compiling all of the trials within a session into a complete serial record of the animals’ performance and stepping through this response data one trial at a time.

Win-stay errors. A “win” was defined as a correct response, while “stay” indicated that the rat responded to the same lever as it did on the previous trial, resulting in an incorrect response. Thus, a win-stay error indicated that the rat responded correctly on the n-1 trial but responded incorrectly on the nth trial.

Lose-stay errors. A lose-stay error indicated that the rat responded incorrectly on the n-1 trial and also on the nth trial by responding on the same lever. Therefore, a lose-stay error represents at least three consecutive responses on the same lever.

Statistical analysis. The data were analyzed via repeated measures analysis of variance (ANOVA) using SPSS for MS Windows and the litter as the statistical unit. Cohort was included as a between-litter variable in all of the analyses to test for possible differences. For the CA task, total errors to criterion were analyzed via repeated measures ANOVA, with exposure group and cohort as between-litter variables and sex as a within-litter variable (i.e., repeated measure). For NCA, proportion correct was analyzed via a four-way repeated measures ANOVA, with exposure group and cohort as between-litter variables and sex and session (1–10) as within-litter variables. For DSA, proportion correct was averaged into five-session block means and analyzed via a four-way repeated measures ANOVA, with exposure group and cohort as between-litter variables and sex and session block (1–5) as within-litter variables. Proportion correct at each delay for DSA was analyzed via a four-way repeated measures ANOVA, with exposure group and cohort as between-litter variables and sex and delay (0, 3, 6, 9, 18) as within-litter variables. Mean correct and incorrect press latencies for NCA and DSA tasks were analyzed using two between-litter variables (exposure and cohort) and two within-litter variables (sex and quartile). For the
DSA response pattern analyses, the number of win-stay and lose-stay errors was analyzed using two between-litter variables (exposure and cohort) and two within-litter variables (sex and block). Only significant (or near-significant, i.e., \( p \leq 0.10 \)) interactions with exposure were further analyzed via simple-effects ANOVA tests (Keppel, 1982). Significance for all analyses was set at \( p \leq 0.05 \).

RESULTS

Reproductive/Developmental Endpoints

The data for the reproductive and developmental outcomes have been published previously (Roegge et al., 2004) and therefore will not be discussed in detail here. In general, the offspring of the PCB- and PCB + MeHg-exposed rats exhibited mild signs of toxicity prior to the beginning of the autoshaping training task, including slightly lower body weights, increased liver- and brain-to-body weight ratio, and a decreased thymus-to-body weight ratio. The offspring of MeHg-exposed rats exhibited no such signs of overt toxicity.

Cued Alternation

Repeated measures ANOVA did not reveal an overall effect of exposure on the number of errors to criterion on CA \( (F(3,33) = 0.542, p = 0.657) \) (see Fig. 1). Similarly, there was no evidence of a sex by exposure interaction \( (F(3,33) = 1.991, p = 0.134) \).

Non-Cued Alternation

Repeated measures ANOVA revealed a significant main effect of exposure on proportion correct \( (F(3,33) = 4.234, p = 0.012) \) as well as a significant exposure by cohort interaction \( (F(6,33) = 3.076, p = 0.017) \). Individual comparisons between exposure groups revealed a significant reduction in proportion correct for the MeHg-only \( (p < 0.001) \) and the PCB + MeHg rats \( (p = 0.017) \) when compared to controls (see Fig. 2A). Individual comparisons between cohorts to investigate the significant exposure by cohort interaction revealed that proportion correct for the PCB-only rats in cohort 3 was significantly lower than either cohort 1 or cohort 2 \((p = 0.026 \text{ and } p = 0.016, \text{ respectively}; \text{see Fig. 2B})\). There were no other significant interactions involving exposure for NCA.

Non-cued alternation lever-press latencies. Analysis of the latencies on correct and incorrect trials during NCA revealed a near-significant overall effect of exposure on correct-trial latencies \( (F(3,33) = 2.812, p = 0.054) \) and a significant overall effect of exposure on incorrect-trial latencies \( (F(3,33) = 4.589, p = 0.009) \). Similarly, there was a near-significant exposure by cohort interaction for both correct and incorrect-trial latencies \((F(6,33) = 2.177, p = 0.070)\) and \((F(6,33) = 2.319, p = 0.056)\) for correct and incorrect trial latencies, respectively). Individual comparisons for correct-trial latencies did not reveal any significant differences between any of the exposure groups and the control group (see Fig. 3A). However, individual comparisons for the incorrect-trial latencies revealed a significant reduction in the latency to press for the PCB + MeHg rats when compared to control, PCB, and MeHg rats \((p = 0.009, p = 0.019, \text{ and } p = 0.021, \text{ respectively})\). Neither the PCB-only \((p = 0.610)\) nor MeHg-only \((p = 0.226)\) rats differed significantly from controls in terms of incorrect press latencies (see Fig. 3B). Further investigation into the near-significant exposure by cohort interactions for both correct and incorrect-trial latencies revealed that the correct-trial latencies for the control group were significantly longer in the third cohort when compared to the second cohort \((3.514 \text{ vs. } 5.0136, \text{ for the second and third cohorts, respectively}; p = 0.023)\). No other exposure group by cohort comparison for either correct- or incorrect trial latencies during NCA proved to be significant.

Delayed spatial alternation. Repeated measures ANOVA revealed a near-significant main effect of exposure on proportion correct on DSA \( (F(3,33) = 2.385, p = 0.087) \). Visual inspection of Figure 4 shows reductions in DSA accuracy for all of the exposed groups when compared to the controls. However, larger error bars for the PCB + MeHg group suggests increased variability. Individual comparisons revealed a significant reduction in the proportion of correct trials for PCB-only \((p = 0.034)\) and MeHg-only \((p = 0.006)\) rats. The reduction in proportion correct for the PCB + MeHg rats was not significant \((p = 0.106)\) due to the aforementioned increase in variability. There were no significant interactions between exposure and any of the

\[ \text{FIG. 1. Total number of errors committed on the cued alternation (CA) task prior to criterion, summed across days and averaged across sex and cohort for control (n = 10), PCB-exposed (n = 13), MeHg-exposed (n = 12), and PCB + MeHg-exposed (n = 10) groups. Error bars represent the SEM.} \]
Because the nature of the impairment was similar on both NCA and DSA, a Pearson $r$ correlation coefficient was calculated to assess whether there was a correlation between overall performance on NCA and overall performance on DSA for each litter, irrespective of exposure group. There was a trend for litters that did better on NCA to do better on DSA.
DSA, but this trend was not statistically significant ($r = 0.278$, $p = 0.065$).

Analysis of proportion correct at each delay averaged across all 25 sessions revealed a near-significant main effect of exposure [$F(3,33) = 2.884$, $p = 0.087$] and a significant exposure by delay interaction [$F(12,132) = 2.307$, $p = 0.011$]. Visual inspection of Figure 5 reveals that the performance of all of the exposed groups was below that of the control group at all but the longest delay. Individual comparisons at each delay revealed significant reductions in proportion correct at all delays except 18 s for the MeHg-only rats when compared to controls ($p = 0.005$, $p = 0.020$, $p = 0.003$, $p = 0.007$, and $p = 0.535$ for 0, 3, 6, 9, and 18-s delays, respectively). The PCB-only rats exhibited significant reductions in proportion correct at 0, 6, and 9 s, but not at the 3 and 18-s delays ($p = 0.045$, $p = 0.113$, $p = 0.032$, $p = 0.027$, and $p = 0.333$ for 0, 3, 6, 9, and 18-s delays, respectively). The PCB + MeHg rats exhibited reductions in proportion correct only at the 0-s delay ($p = 0.001$, $p = 0.085$, $p = 0.132$, $p = 0.199$, and $p = 0.955$ for 0, 3, 6, 9, and 18-s delays, respectively).

Repeated measures ANOVA of errors by quartile by five-session block was conducted to examine the possibility that the increase in errors for the exposed rats was due to late session reductions in accuracy. No significant differences were observed between groups when performance was examined by quartile of session, indicating that all rats performed similarly throughout the entire test session (data not shown).

**DSA Lever-Press Latencies**

Repeated measures ANOVA of average correct- and incorrect-trial lever-press latencies during DSA testing did not reveal an overall effect of exposure ($F(3,33) = 0.571$, $p = 0.638$) and [$F(3,33) = 0.092$, $p = 0.964$] for correct- and incorrect-trial latencies, respectively. There was, however, a significant effect of cohort for both correct- and incorrect-trial latencies ($F(2,33) = 6.748$, $p = 0.003$) and [$F(2,33) = 4.439$, $p = 0.020$] for correct- and incorrect-trial latencies, respectively. Individual comparisons between cohorts revealed that the overall cohort effect was due to significantly longer latencies in the third cohort relative to the first and second cohorts for both correct and incorrect trial latencies. There were several significant or near-significant interactions for the correct-trial lever-press latencies including a significant exposure by cohort by sex [$F(6,132) = 2.408$, $p = 0.049$] and exposure by cohort by sex by block [$F(24,132) = 1.701$, $p = 0.031$] interaction, and a near-significant exposure by sex by block [$F(12,132) = 1.811$, $p = 0.052$] interaction. However, these interactions do not appear to be due to an interaction with exposure, but rather due to an interaction with sex. When sex is removed from the analysis, the interactions are no longer significant. Examination of the data (data not shown) revealed that males tended to maintain a consistent press latency on correct trials across sessions while females tended to exhibit increased latencies across sessions and this is evidenced by a significant sex by block [$F(4,132) = 22.454$, $p < 0.001$] and sex by block by cohort interaction [$F(8,132) = 3.607$, $p = 0.001$].

**DSA Response Pattern Analyses**

Analysis of “win-stay” errors was conducted to assess whether the exposed rats were more likely to be influenced...
by recent reinforcement history and return to the same lever upon which reinforcement was just received rather than respond according to the alternation contingencies. Although there seemed to be a trend for the exposed rats to exhibit more of this type of error (see inset of Fig. 6A), repeated measures ANOVA did not reveal any significant differences between the exposed groups [\(F(3,33) = 1.915, p = 0.146\)] (Fig. 6A). Visual inspection of Figure 6A indicates that all groups began testing with a similar number of win-stay errors, and the reduction in errors was more pronounced in the control group relative to the exposed groups. However, none of the repeated measures interactions with exposure were significant.

The tendency for the rats to perseveratively respond to the incorrect lever in spite of repeated nonreinforcement was investigated by examining the number of errors committed on the same lever after the first error had been committed (i.e., “lose-stay” errors). Repeated measures ANOVA revealed a significant effect of exposure [\(F(3,33) = 2.888, p = 0.05\)] and a near-significant effect of cohort [\(F(2,33) = 2.643, p = 0.086\)]. Additionally, there was a significant exposure by block interaction [\(F(12, 132) = 1.823, p = 0.05\)] and near-significant exposure by sex interaction [\(F(1, 33) = 2.779, p = 0.056\)]. All exposure groups showed increased perseverative errors relative to controls (see inset of Fig. 6B). Individual comparisons between exposure groups at each five-session block revealed that the MeHg-only rats exhibited an increase in perseverative errors when compared to controls across all of the session blocks with the exception of the third block (\(p = 0.011, 0.002, 0.024, 0.024\) for blocks 1, 2, 4, and 5, respectively; see Fig. 6B). Rats exposed only to PCBs exhibited an increase relative to controls in the later blocks (\(p = 0.004\) and 0.041 for blocks 4 and 5, respectively) while rats exposed to both PCB and MeHg exhibited increases in perseverative errors relative to controls in the earlier blocks (\(p = 0.001\) and 0.046 for blocks 1 and 2, respectively).

DISCUSSION

The purpose of the current study was to determine if combined developmental exposure to PCBs and MeHg would potentiate the impairments induced by PCBs alone on a series of spatial alternation tasks. Gestational and lactational exposure to the commercial PCB mixture Aroclor 1254 (A1254; PCB-only group) resulted in a significant reduction in proportion correct on a delayed spatial alternation task (DSA). The PCB-only rats did not exhibit a significant reduction in performance on either of the two “training” procedures, cued alternation (CA) or non-cued alternation (NCA). Exposure to MeHg alone resulted in significant performance reductions on both the NCA and DSA tasks, but not the CA task. Combined exposure to PCB + MeHg also caused reductions in NCA and DSA performance, but the PCB + MeHg rats performed no worse than rats exposed to PCBs or MeHg alone. Response latencies were unaffected by

---

**FIG. 6.** Mean number of “win-stay” (A) and “lose-stay” (B) errors committed during the delayed spatial alternation (DSA) task averaged across sex, delay, and cohort for each 5-session block for control (\(n = 10\)), PCB-exposed (\(n = 13\)), MeHg-exposed (\(n = 12\)), and PCB + MeHg-exposed (\(n = 10\)) groups. Note: “a,” “b,” or “c” denotes a statistically significant difference from the control group for the PCB-, MeHg-, or PCB + MeHg-exposed group, respectively (\(p < 0.05\)). Inset graphs represent mean number of “win-stay” (A) and “lose-stay” (B) errors committed per session averaged across sex, delay, session block, and cohort for control (\(n = 10\)), PCB-exposed (\(n = 13\)), MeHg-exposed (\(n = 12\)), and PCB + MeHg-exposed (\(n = 10\)) groups. Error bars represent the SEM. Note: * in the inset graphs denotes statistically significant difference from control group (\(p < 0.05\)).
exposure in both the CA and DSA task, but there was evidence of an interaction between PCBs and MeHg on the NCA task for incorrect trial press latencies with PCB + MeHg-exposed animals exhibiting significantly shorter response latencies than the other groups. Analysis of DSA performance as a function of delay revealed similar deficits for all three exposure groups across all but the longest delay suggesting that the observed deficit was not an impairment of spatial memory, but rather was indicative of a possible associative or attentional impairment. Furthermore, analysis of perseverative errors revealed that all of the exposed groups exhibited a greater tendency to emit these types of errors. Taken collectively, the similarities in the nature of the impairment across the PCB and MeHg exposure groups suggest a similar site of action or neurotoxicity.

Non-Cued Alternation (NCA) Impairments

The purpose of the NCA task was twofold. The first was to train the rats to alternate their responses between two spatial locations (i.e., left or right) without the benefit of a visual cue (as was the case in the CA task). The second was to allow for the assessment of alternation performance in which the memory requirement was minimized since no delays were used during this condition. Presumably then, optimal performance on NCA was primarily dependent on the ability of the rat to effectively attend to and encode its own behavior and use that information to guide its next response. Rats exposed to MeHg-only and the combined PCB + MeHg performed more poorly on this task, as evidenced by a significantly lower proportion of trials correct.

The response latency data from the NCA task revealed significantly shorter response latencies on incorrect trials for the combined PCB + MeHg group that were not evident in the PCB-only or MeHg-only groups, suggesting an interactive effect of PCBs and MeHg on this measure. The mechanism through which PCBs and MeHg might interact to cause shorter response latencies is unknown, but a dopaminergic hypothesis has been postulated that relates changes in dopamine with changes in the reinforcer strength gradient predisposing the organism to shorter response latencies and response bursts (Sagvolden et al., 1996). The resulting behavioral condition is similar to Attention Deficit Hyperactivity Disorder (ADHD). Animal studies have shown that both PCBs and/or MeHg can alter brain dopamine concentrations in vitro and in vivo (Bemis and Seegal, 1999; Faro et al., 1997; Seegal et al., 1997). Evidence from human and animal studies of PCB (Berger et al., 2001; Jacobson and Jacobson, 2003; Rice, 1998; Stewart et al., 2003a) and MeHg (Gilbert et al., 1996; Grandjean et al., 1997; Rice, 1992) exposure suggests that these contaminants have the ability to alter attention and/or response inhibition. However, given the fact that overall NCA performance was not significantly reduced in the PCB + MeHg group, the relevance of the decrease in incorrect response latencies is unclear.

The impairment on NCA following developmental MeHg exposure was surprising because to date there have been few demonstrations of cognitive impairments in rats following developmental exposure to MeHg. Bornhausen et al. (1980) and Newland and Rasmussen (2000) both reported that MeHg-exposed rats exhibited alterations in DRH responding. However, the effect of MeHg was small, and in the Newland and Rasmussen (2000) study, a deficit did not become evident until the rats were aged, suggesting that the cognitive effects of MeHg are subtle and require other challenges (i.e., aging) to the organism. An alternative explanation is that a MeHg-induced motor impairment may have contributed to the deficits seen on this task. However, this seems unlikely given that the reductions in DRH reinforcement rate in MeHg-exposed rats were not due to a disruption of the required response sequence (which would be indicative of motor impairment), but rather to increased pausing between response sequence bouts.

Even in studies utilizing monkeys, cognitive deficits following developmental MeHg exposure have not been consistently demonstrated. In monkeys that received MeHg during perinatal development at levels high enough to cause sensory and somatosensory impairments (Rice and Gilbert, 1990, 1995), cognitive impairment was restricted to deficits in temporal discrimination evidenced by earlier responding on a FI schedule of reinforcement (Rice, 1992). Indeed, some of these same monkeys were actually slightly better than control monkeys when tested on a visual reversal learning (RL) task (Rice, 1992). As was the case with the PCB + MeHg rats on NCA performance in the current study, the shorter response latencies exhibited during FI schedule testing in the Rice (1992) study were not predictive of impairments in other cognitive domains (i.e., reversal learning). In a separate series of experiments, MeHg-induced impairments were observed in infant monkeys on object permanence (Burbacher et al., 1986), visual recognition memory (Gundersen et al., 1988), and FI schedule performance (Gilbert et al., 1996), but these monkeys were not impaired on DSA (Gilbert et al., 1993).

The reduction in performance on NCA for the groups receiving dietary MeHg suggests that: (1) the low dose of MeHg used in the current study was sufficient to produce clear cognitive alterations in the rat offspring; (2) the addition of PCBs exacerbated the trend for shorter incorrect response latencies in the MeHg-exposed rats; (3) the addition of PCBs did not potentiate any of the observed impairments on measures of overall performance; and (4) the MeHg-induced cognitive deficit was likely associative or attentional rather than mnemonic.

Delayed spatial alternation (DSA) impairments. The DSA task allows for the assessment of learning and memory within the same task. Developmental PCB exposure has been linked with childhood memory deficits in human epidemiology studies (see Durvill et al., 2000; Jacobson et al., 1985, 1990, 1992), and the question of whether PCBs and/or MeHg alter an organism’s
ability to retain information in memory can be assessed using the DSA task. Although the link between memory impairment and developmental MeHg exposure has yet to be firmly established in human studies, deficits in object permanence (Burbacher et al., 1986) and visual recognition memory (Gunderson et al., 1988) in monkeys developmentally exposed to MeHg demonstrate the ability of MeHg to alter memory function in primates.

Examination of Figure 5 reveals that, when performance was analyzed as a function of delay, the decrease in performance for the exposed rats was, in general, consistent across all of the delays except for the longest delay, at which point the performance of all the rats dropped to chance levels, signifying that 18 s approximates the upper limit of the rat’s ability to hold response position information in working memory for the current task. If spatial memory was impaired following PCB and/or MeHg exposure, performance would decrease more rapidly as the delay increased. This pattern of results was not observed, again suggesting that factors other than memory are the cause of the decline in DSA performance.

A similar pattern of effects has been demonstrated previously in PCB-exposed monkeys (Levin et al., 1988; Rice and Hayward, 1997) and PCB-exposed rats (Schantz et al., 1995). Perinatal exposure of monkeys to commercial PCB mixtures (Levin et al., 1988) or postnatal exposure to a PCB mixture representative of the congeners found in human breast milk resulted in reductions in overall DSA performance (Rice and Hayward, 1997). For both of these studies, when performance was analyzed across delay, similar reductions were observed across delays, suggesting that the impairment was not the result of reduced memory ability. Previous authors interpreted the effect as the result of reduced attentiveness (Levin et al., 1988) or as a learning/performance decrement (Rice and Hayward, 1997). Similarly, perinatal exposure of rats to the ortho-substituted PCB congeners 28, 118, or 153 caused DSA impairments in female offspring that were similar to that found in the aforementioned monkey studies; the PCB-exposed female rats were impaired relative to controls at all delays, and this difference did not increase with an increase in delay (Schantz et al., 1995). In contrast to PCBs, MeHg exposure has not been shown to affect DSA performance in monkeys (Gilbert et al., 1993).

In addition to the traditional measures of DSA performance (e.g., proportion correct), the tendency to perseveratively respond was analyzed in the current study through the assessment of “win-stay” and “lose-stay” errors. All of the exposed animals exhibited an increased tendency to emit “lose-stay” errors, indicating that these animals were more likely to emit strings of consecutive errors rather than alternate their responses following the first error. This type of error pattern is suggestive of a reduced associative ability in that there is less sensitivity to the consequences of an animal’s own behavior. A similar effect has been demonstrated previously in monkeys postnatally exposed to PCBs (Rice and Hayward, 1997).

Lastly, exposure to PCBs and/or MeHg did not impair the rate at which performance improved on DSA. Upon switching from NCA to the DSA task, the exposed rats began at a lower performance level than did the controls, and this difference was maintained throughout DSA testing (as evidenced by a lack of a significant treatment by block interaction). This suggests that exposure to PCBs and/or MeHg caused impairments that were evident early in learning, but exposure did not impair the rate at which performance improved.

The possible role of attention to behavior in spatial alternation performance. That attention to self-initiated behavior may be affected in rats developmentally exposed to PCBs and/or MeHg is supported by the lack of an effect on CA performance, during which a visual cue signals the correct spatial location. During CA, the rat learned to track the visual stimulus in order to satisfy the performance criterion of this task. Upon completion of the CA task, the visual stimulus was removed, and optimal performance on NCA was then dependent on the rat attending to where it last pressed so that it could use that information to guide future behavior. When the visual cue was removed and optimal performance became more dependent on attention to behavior, all of the exposed groups suffered a greater loss in accuracy than did controls, although the PCB-only group was not statistically different from control rats.

Research by Bushnell and colleagues suggests that developmental exposure to PCBs does not affect sustained attention in rats. When a visual stimulus was briefly increased in luminance above the background light levels, rats developmentally exposed to either coplanar PCB congeners (Bushnell and Rice, 1999) or A1254 (Bushnell et al., 2002) were as able as nonexposed controls to attend to the visual stimulus and correctly detect when a signal was presented. However, the attentional requirements for optimal NCA and DSA performance are quite different from those of a sustained attention procedure and require the ability to selectively attend to a stimulus (or event) in the presence of competing stimuli.

Optimal performance on any given trial during NCA or DSA testing requires that the organism attend to its own behavior as it presses a lever, encode and/or remember to which lever the response was allocated, and use that information to respond on the next trial. However, the moment the rat emits a response it is confronted with a competing stimulus to which it can attend: the presence or absence of the feeder being activated. This temporal relationship between the lever press and reinforcement is necessary to ensure that the act of lever pressing is reinforced (rather than “other” behavior). However, it could have the unfortunate side effect of diverting attention away from the rat’s own behavior (i.e., which lever was pressed?) to the consequences of that behavior (i.e., will the lever press result in reinforcement?). So, even though the rat is “overtly” responding to the response lever, it may be “covertly” attending to the possibility of
feeder activation. This is referred to as “expectancy” (see Bushnell, 1998) in that the organism is more likely to attend to the more salient event of reinforcement. If the rat’s attention is more focused on the expected outcomes of its behavior rather than the stimuli that predict reinforcement, the rat may find itself unable to recall the previous response. While it is difficult to speculate why a rat would attend more to response expectancies than to the response itself, one possibility is that these rats are more emotionally reactive and motivated for the expectancies than to the response itself. Thus, altering their response expectancies, one possibility is that these rats are more emotionally reactive and motivated for the reinforce value. The expected result of such a change would be an increase in impulsivity and presumably a greater focus of attention on behavioral outcomes rather than the behavior itself.

A rat exhibiting impairments in attention as described above would be expected to perform more poorly in an operant version rather than a T-maze version of DSA, because the spatial location of the response alternatives and the associated spatial cues in the operant analogue are not very disparate, the responses themselves are quite homogeneous in nature (i.e., one response is like every other response that has occurred), and the response and reinforcement occur concurrently. In a T-maze version of DSA, the responses are much more spatially disparate (they can be separated by as much as one meter), the external cues associated with each spatial location are often different, the time to execute the response is much longer and more effortful (thus making the spatial memory more distinctive), and reinforcement occurs at the spatial location (i.e., the rat is allowed to consume the reinforcer while at the correct spatial location), all of which have the effect of distinguishing the response from the consequences of the response. Therefore, the T-maze DSA task would be expected to be much easier than its operant analogue. If PCBs and/or MeHg exerted cognitive effects via disruption of selective attention, DSA impairments might not be expected on T-maze analogues of the task.

The data from studies utilizing a T-maze to assess DSA performance following perinatal PCB exposure, in general, support the contention that deficits are less likely to be observed in T-maze analogues of the task. In Schantz et al. (1995), deficits on the T-Maze DSA task were observed only in the most highly exposed female rats (32 mg/kg/day of PCB 28, 16 mg/kg/day of PCB 118, or 64 mg/kg/day of PCB 153 from GD 10–16). Males were unaffected, even at the highest doses. Similarly, rats exposed to a dose of Aroclor 1254 similar to that used in the current study did not exhibit any impairments on a T-maze DSA task (Zahalka et al., 2001). If exposure to PCBs and/or MeHg causes subtle DSA impairments via the disruption of selective attention, the T-Maze DSA task may not be sensitive enough to reliably detect an impairment.

Summary

The reduction in performance on NCA and/or DSA for the groups receiving PCBs, MeHg, or PCB + MeHg suggests that (1) developmental exposure to PCBs and/or MeHg is capable of impairing spatial alternation performance; (2) the nature of the DSA impairment does not appear to be related to reductions in working memory, but possibly to alterations in associative ability or attention; and (3) the combination of PCBs and MeHg exacerbated the trend for shorter response latencies on NCA, but there was no evidence for the combination of PCBs and MeHg to potentiate any observed impairments on measures of overall performance.

REFERENCES


