COGNITIVE, MOTOR AND AUDITORY DEFICITS RESULTING FROM EXPOSURE TO PCBS AND METHYL MERCURY DURING EARLY DEVELOPMENT

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Introduction

Polychlorinated biphenyls (PCBs) and methyl mercury (MeHg) are widespread environmental contaminants that are neurotoxic in laboratory animals and humans[1]. Both chemicals accumulate in aquatic ecosystems creating the potential for combined exposure in humans and wildlife consuming fish, seafood or marine mammals from contaminated areas. Recent *in vitro* studies suggest that PCBs and MeHg may have additive or interactive effects on nervous system function[2,3]. Additional studies are needed to determine whether the two chemicals interact to produce functional deficits *in vivo*. Accordingly we designed experiments to determine whether combined exposure to PCBs and MeHg during gestation and lactation would result in larger deficits in cognitive, motor or auditory function than exposure to either chemical alone.

Methods and Materials

Female Long Evans rats (Harlan Sprague Dawley; Madison, WI) were housed in a temperature and humidity controlled room (22^{o} C, 40-55% humidity) in plastic rodent cages with corn-cob bedding. Food and water were available *ad libitum*. Chemical exposure began four weeks prior to mating and continued through postnatal day (PND) 16. PCB-exposed females were fed one half of a vanilla wafer cookie containing 6 mg/kg Aroclor 1254 (Lot #124-191; Accustandard, New Haven, CT) dissolved in corn oil vehicle. MeHg-exposed females received drinking water adulterated with 0.5μg/ml methylmercuric chloride (Alfa Aesar Chemicals). Water bottles were weighed daily to monitor intake. In previous studies these doses of PCBs and MeHg resulted in subtle but lasting deficits in behavioral functioning[4,5]. Animals in the combined exposure group received both the PCB containing cookies and the MeHg adulterated water. Control females were fed cookies containing only the corn oil vehicle and received unadulterated tap water.

For breeding each female was placed with an unexposed male daily for up to one week during the dark phase of the diurnal cycle. Pregnancy was determined by the presence of a sperm plug. On PND 2 litters were reduced to 10 pups (5 males and 5 females). Extra pups from large litters were cross fostered within the same exposure group as needed. Pups were weaned on PND 21 and housed in same-sex, same-exposure pairs. All procedures were performed in AAALAC approved facilities according to protocols approved by the Institutional Animal Care and Use Committee.

As adults, one male and one female from each litter were tested on several tests of balance and coordination, including a rotating rod task in which rats were required to cross a 2-meter long rotating rod to reach a platform. The rats received three consecutive days of training. On the first day a rod with a rough, sandpaper like surface was used. On the second day a smooth rod with two foam hurdles located 1/3 and 2/3's of the way across was used and on the final day of testing a

smooth rod with no hurdles was used. On each day of testing the rats received 3 consecutive trials at each of five rotation speeds: 0, 10, 15, 25 and 30 rpm. The time to traverse the rod and the number of slips were recorded on each trial. Testing was terminated for the day if a rat made more than 5 slips on one trial. Unfinished trials were assigned the maximum score of 5 slips.

Another male and female from each litter were tested on a battery of cognitive tests in standard, commercially available operant testing chambers (Med Associates, St. Albans, VT) with retractable response levers. Rats were shaped to press the response levers using an autoshaping procedure, followed by additional lever press training. They were then trained on a series of cognitive tasks including a test of spatial working memory known as delayed spatial alternation (DSA). Each test session lasted for 200 trials or 90 min; whichever occurred first. The rats were first trained on a cued alternation task in which they were trained to press the lever opposite that selected on the previous trial. At the beginning of each trial the levers were extended into the chamber and the light over the correct lever was illuminated to signal the correct response. Next the rats were trained on a non-cued alternation task, in which they were still required to alternate between the two levers, but the cue lights no longer signaled the correct lever. Lastly the rats were tested on the DSA task. DSA was identical to non-cued alternation except that variable delays of 0, 3, 6, 9, or 18 seconds were randomly imposed between the trials, with the stipulations that the number of trials at each delay was balanced within each test session and that a particular delay was not presented on more than three consecutive trials. The rats received 25 sessions of DSA testing.

After the cognitive testing was completed the same rats underwent auditory testing. Distortion Product Otoacoustic Emissions (DPOAEs) were recorded using Tucker Davis Technologies (TDT, Florida) System 2 / System 3 digital signal processing hardware and software. The DPOAE stimuli were presented and recorded using two programmable attenuators, a single probe unit which contained two Etymotic ER-2 earphones and one Etymotic ER10B ear canal microphone, and an amplifier that provided 45 dB of gain to the DPOAE responses prior to digital conversion. Rats were sedated with 0.45 ml/kg ketamine/xylazine (87:13) prior to DPOAE testing. The DPOAE stimuli were generated by simultaneously presenting two sinusoids differing in frequency (the lower frequency labeled f_1 and the higher frequency f_2) into the sealed ear canal. The latency and amplitude of the $2f_1 - f_2$ distortion product were measured by recording the pressure in the sealed ear canal. Seven stimulus pairs were selected for DPOAE testing which included f_2s = 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 KHZ (f_2/f_1=1.2). The sound levels for the f_1 and f_2 primaries were calibrated to 60 dB SPL and 50 dB SPL, respectively.

The motor, cognitive and auditory data were analyzed via repeated measures analysis of variance. The litter was the unit of variance in all analyses and sex was nested within litter. Significance was ascribed at $p < 0.05$.

Results and Discussion

The rotating rod task measured balance and coordination. Statistical analysis of the number of slips rats made while crossing the rod revealed a significant exposure by rpm interaction ($p=0.002$). Comparisons of each exposure group to the control group at each of the different rotation speeds revealed that the rats exposed to both PCBs and MeHg slipped more than the controls at both 25 and 30 rpm (Figure 1). PCBs and MeHg seemed to have an additive effect since neither chemical alone caused a significant number of slips. However, visual inspection of

the data suggest that PCB exposure contributed more to the deficit than did MeHg. The sex by exposure and sex by exposure by rpm interactions were not significant, indicating that males and females were similarly affected by the combined PCB and MeHg exposure.

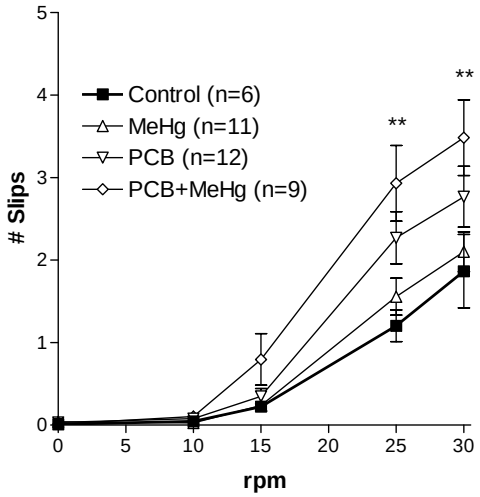

Figure 1. Mean number of slips (± SE) on the rotating rod averaged across three days of testing. PCB+MeHg rats made significantly more slips than controls at 25 and 30 rpm (** $p < 0.01$).

The delayed spatial alternation test measured spatial working memory. Statistical analysis revealed a nearly significant main effect of exposure on the proportion of correct responses (p=0.087). Comparisons of the exposed groups to the control group revealed that all three exposed groups made fewer correct responses than the controls (Figure 2a). Males and females were similarly affected by exposure. However, the combined exposure group did not do more poorly than the groups exposed to either PCBs or MeHg alone, suggesting that the two compounds do not have additive effects on spatial learning and memory. Analysis of the proportion correct at each delay revealed a significant exposure by delay interaction (p=0.011). The proportion correct for the exposed groups was lower than that for the control group at all but the longest delay (Figure 2b). Given that differences were present even when there was no delay between trials and that the deficit did not increase as the length of the delay increased it is likely that the decrement in performance in the exposed groups was associational or attentional rather than mnemonic.

Decreased DPOAE amplitudes and elevated DPOAE thresholds indicate hearing loss related to damage to the outer hair cells of the cochlea. Statistical analysis of the DPOAE results revealed significant main effects of exposure for both DPOAE amplitude (expressed as signal to noise ratio;p=0.01) and threshold (p=0.002) Comparisons of the exposed groups to the control group indicated that exposure to PCBs alone or PCBs and MeHg reduced the signal to noise ratio across all frequencies tested, with significant reductions at 2, 3,4, and 6 KHz (Figure 3a). DPOAE thresholds were increased across all frequencies, with significant differences at 3, 4, 6 and 12 KHz. MeHg alone did not alter DPOAE amplitudes or thresholds, and exposure to MeHg did not exacerbate the effects of PCBs. These results confirm earlier reports of PCB-related hearing loss[6] and implicate the cochlea as a primary site of damage.

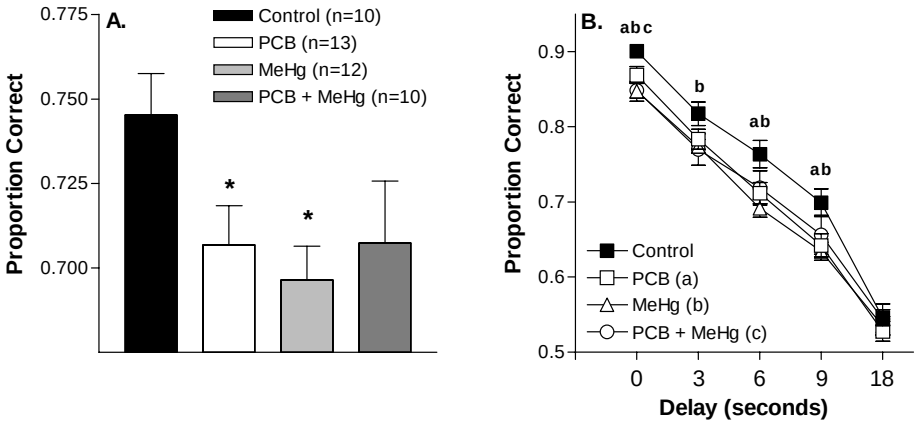

Figure 2. Mean proportion correct (±SE) on DSA averaged across all delays (A) and at each individual delay (B). All exposed groups had lower proportions correct than controls (*p<0.05).

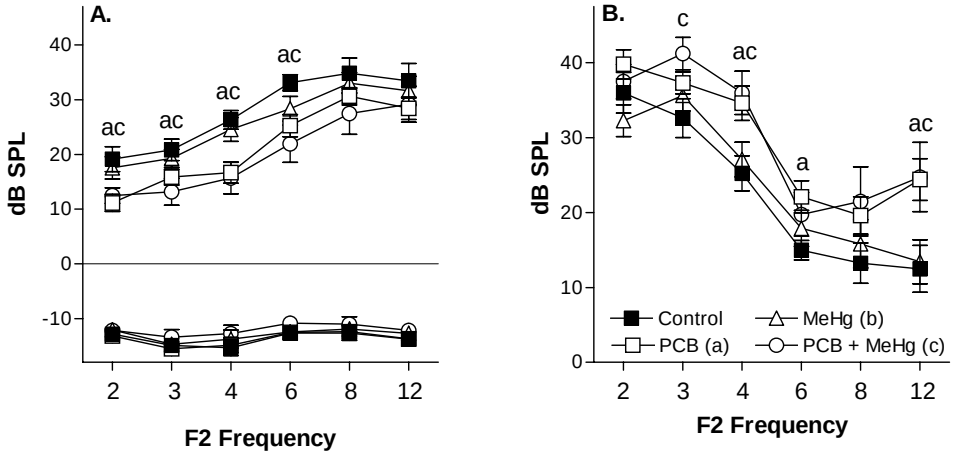

Figure 3. DPOAE signal to noise ratios (A) were reduced and thresholds (B) were increased across all frequencies in the PCB and PCB+MeHg groups (a and c indicate p <0.05).

References

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