Effects of Developmental Exposure to PCBs and Methylmercury on Auditory Function

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Introduction

Both polychlorinated biphenyls (PCBs) and methylmercury (MeHg) are ubiquitous environmental contaminants that co-localize in aquatic ecosystems where they enter and bioaccumulate in the food chain. Consumption of contaminated fish represents the primary source of PCB and MeHg exposure for humans and wildlife. Both toxicants can reach a developing child through in utero or lactational exposure.

Recent research has shown that hearing impairments can result from developmental exposure to either PCBs or MeHg[1,2,3,4,5]. In rats, PCBs appear to disrupt the normal development of the cochlea by inhibiting thyroid hormone during the period when low frequency hearing is developing[1,2,3]. Thus, developmental exposure to PCBs affects primarily low frequency hearing (< 1kHz)[1,2,3]. A cochlear site of action is further supported by our recent research showing compromises in the mechanical integrity of the cochlea of PCB-exposed rats through the measurement of distortion product otoacoustic emissions (DPOAEs)[4]. Developmental exposure to MeHg has also been shown to impair hearing in monkeys, but the impairments were only observed from the middle to the highest frequencies tested (10 – 12.5kHz and 25kHz)[5].

Because PCBs and MeHg often occur together in the environment, additional studies are needed to determine whether the two chemicals interact to produce auditory deficits. Accordingly, we designed experiments to determine whether combined exposure to PCBs and MeHg during gestation and lactation would result in larger deficits in auditory function than exposure to either chemical alone. The auditory tests we utilized are particularly valuable because the same tests can be used to test auditory function of human infants that may have been exposed to PCBs and MeHg during development.

Methods

Animals, Exposure, and Mating

Sixty-three primiparous female Long-Evans rats, approximately 60 days old, were obtained from Harlan (Madison, WI) for dosing and mating. Beginning one week after their arrival at the University of Illinois, the females were weighed and dosed daily for 66 consecutive days. PCB-exposed females were fed one-half of a Keebler Vanilla Wafer cookie onto which 6 mg/kg Aroclor 1254 (Lot #124-191; AccuStandard, New Haven, CT) dissolved in corn oil vehicle was

pipetted. MeHg-exposed females received continuous access to drinking water into which methylmercuric chloride (Alfa Aesar Chemicals) was dissolved at a concentration of 0.5 µg/ml (0.5 ppm). PCB + MeHg-exposed females were fed the 6 mg/kg A1254 contaminated cookies and had the continuously available 0.5 ppm MeHg adulterated drinking water in their home cages. Control females were fed vanilla wafer cookies that contained only the corn oil vehicle and received unadulterated tap water. Twenty-eight days after the beginning of dosing, all females were mated with unexposed male Long Evans rats. Dosing continued until PND 16. On PND 21 the pups were weaned and one male and one female pup from each litter were selected randomly and tested on a battery of cognitive and sensory tests. Distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) testing began at approximately 600 days of age.

Apparatus

Auditory Brainstem Responses (ABRs) and Distortion Product Otoacoustic Emissions (DPOAEs) were recorded using Tucker Davis Technologies (TDT, Florida) System 2 / System 3 digital signal processing hardware and software. The ABR stimuli were presented using a programmable attenuator and a TDT EC-2 speaker. The ABR responses were recorded using needle electrodes connected to a DB4 headstage that amplified the analog voltage recordings 60,000 times and band pass filtered them with 3 dB cutoffs at 100 Hz and 3 KHz before being digitally converted. The instrumentation used to present the DPOAE stimuli and record the responses consisted of 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.

Procedure

All of the rats were sedated with 0.5 ml/kg ketamine/xylazine (87:13) ip prior to ABR testing. The ABR stimulus was a 65 dB peSPL sinusoid presented at a rate of 21.8/s. The frequencies selected for testing included 0.75, 1.5, 3.0, 6.0, 12.0, 24.0, and 48.0 kHz and were presented sequentially beginning with the lowest frequency. Stimulus duration was 6 ms for the 0.75 kHz, 3 ms for the 1.5 kHz, and 1.5 ms for the frequencies between 3.0 and 48.0 kHz. A TDT EC-2 speaker was used to present the ABR stimuli into the sealed ear canal of the rat using a 15 cm piece of surgical tubing. The ABR responses were differential voltage recordings from needle electrodes placed under the skin on the scalp at the vertex (noninverting electrode) and ipsilateral mastoid (inverting electrode). The ground electrode was placed on the back of the neck. Each ABR response represents the average responses to 500 stimulus presentations. The latency and amplitude (peak to following trough) of each of the first four positive peaks (Peaks I-IV) were measured. Peak II was the most robust and easily identifiable peak, and was, therefore, analyzed for alterations in ABR latency and amplitude.

Immediately following ABR testing, the rats underwent 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 of the rat. 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 DPOAE signal to noise ratios were calculated by subtracting the $2f_1$- f_2 DPOAE levels from the surrounding noise levels. The noise

was defined as the average of the 10 neighboring frequencies, 5 above and 5 below the $2f_1$- f_2 distortion product. DPOAE thresholds were determined by reducing the f_1 and f_2 primaries in 5 dB steps and were defined as the highest dB level in which the $2f_1$- f_2 distortion product was no longer greater than 6 dB above the surrounding noise.

Statistical Analysis

The 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. If a litter was incomplete due to the loss of an animal, the surviving animal served as the mean for that litter. Significance was ascribed at $p < 0.05$.

Results and Discussion

Decreases in DPOAE signal to noise ratios and elevations in DPOAE thresholds are indicative of damage to the outer hair cells of the cochlea. Exposure to PCBs appears to impair the normal development of the cochlea through the disruption of thyroid hormone, which is critical for normal cochlear development[1,2,3]. Statistical analysis of the signal to noise ratio for all of the frequencies between 2kHz and 12kHz revealed a significant main effect of exposure ($p = 0.01$). Individual comparisons at each frequency revealed significant reductions in signal to noise ratio at 2, 3, 4, and 6 kHz for the PCB-exposed and PCB + MeHg-exposed rats (Figure 1A). Analysis of the DPOAE thresholds between 2kHz and 12kHz revealed a significant main effect of exposure ($p = 0.002$). Individual comparisons at each frequency revealed that PCB-exposed and/or PCB + MeHg-exposed rats exhibited a significant elevation in threshold at 3, 4, 6 and/or 12kHz (Figure 1B). These data show that the effects of PCBs on DPOAE signal to noise ratio and threshold extend to frequencies higher than those previously demonstrated using reflex modification audiometry (i.e., < 1kHz)[2], suggesting that the auditory effects of PCBs may extend to higher frequencies than previously believed. Developmental exposure to MeHg alone did not alter either DPOAE signal to noise ratios or DPOAE thresholds. Combined exposure to PCB + MeHg did not potentiate the hearing deficits seen in the rats exposed to PCBs alone.

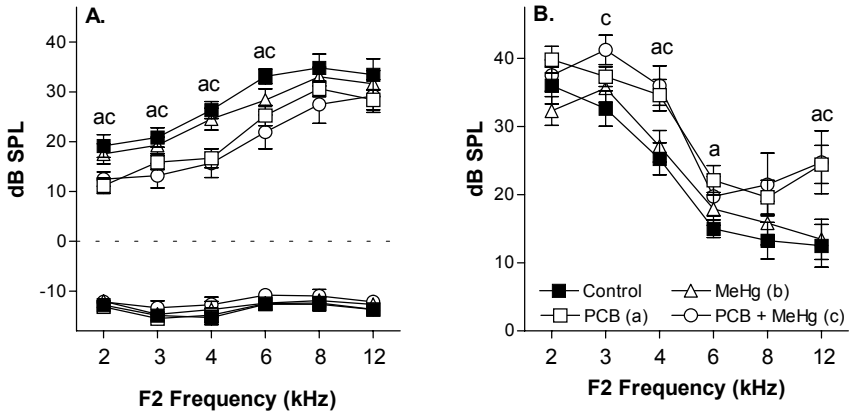

Figure 1. DPOAE signal to noise ratios (A) were reduced and thresholds (B) were increased across all frequencies in the PCB and PCB + MeHg groups (a and c indicate $p<0.05$). Absolute noise levels are presented in the bottom portion of Figure 1A.

Alterations in ABR amplitudes and latencies are indicative of damage along the auditory pathway. Developmental exposure to PCBs has been shown to reduce ABR amplitudes at 4 kHz and 16 kHz[3]. Statistical analysis of Peak II latencies between 3 kHz and 48 kHz did not reveal a significant main effect of exposure ($p = .302$). Analysis of Peak II amplitudes did not reveal a significant main effect of exposure ($p = .278$), but there was a significant exposure by frequency interaction ($p < 0.001$). Individual comparisons at each frequency between 3 kHz and 48 kHz revealed significant reduction at 12 kHz for the PCB-exposed rats and a near significant reduction at 6 kHz for the PCB-exposed rats. Neither the MeHg-exposed nor the PCB + MeHg-exposed rats exhibited any significant amplitude reductions at any of the analyzed frequencies. Because the auditory effects of MeHg are believed to be centrally mediated (i.e., auditory nerve, cortical nuclei, etc.), the possibility exists that MeHg exposure may affect later ABR peaks. The ABR amplitude data are consistent with the results from DPOAE testing which demonstrate the potential for PCBs to alter auditory function. Exposure to MeHg did not potentiate the reductions seen in the PCB alone group.

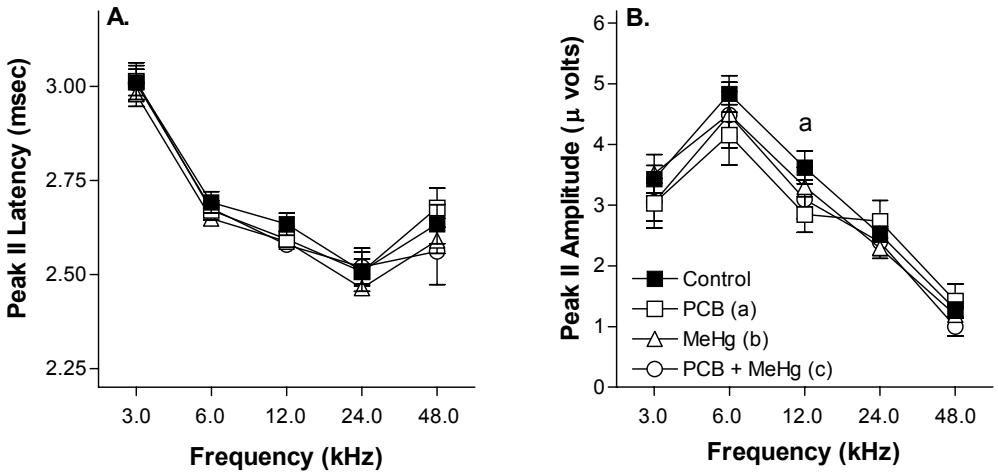

Figure 2. ABR Peak II latencies (A) are not altered following exposure to PCBs and/or MeHg, but Peak II amplitudes are significantly reduced in rats exposed to PCBs only (a indicates $p<0.05$).

References

1. Crofton, K.M., Ding, D.L., Padich, R., Taylor, M., and Henderson, D. (2000) Hearing Research 144, 196-204.
2. Goldey, E.S., Kehn, L.S., Lau, C., Rehnberg, G.L., and Crofton, K.M. (1995) Toxicol. App. Pharmacol. 165, 77-88.
3. Herr, D.W., Goldey, E.S. and Crofton, K.M. (1996) Fundam. Appl. Toxicol. 33, 120-128.
4. Lasky, R.E., Widholm, J.J., Crofton, K.M., and Schantz, S.L. (2002). Toxicol. Sci. 68, 458-464.
5. Rice, D.C., and Gilbert, S.G. (1992) Toxicol. App. Pharmacol. 115, 6-10.