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Developmental PCB exposure increases susceptibility to audiogenic seizures in adulthood

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ABSTRACT

Developmental exposure to polychlorinated biphenyls (PCBs) causes auditory deficits. Thus, we recently conducted a study to investigate if developmental PCB exposure would exacerbate noise-induced hearing loss in adulthood. Unexpectedly, some PCB-exposed rats exhibited seizure-like behaviors when exposed to loud noise. Therefore, we conducted the current experiment to determine if adult rats perinatally exposed to PCBs are more susceptible to audiogenic seizures when tested in a standard audiogenic seizure paradigm. Adult male and female rats exposed to PCBs during gestation and lactation (0, 1, 3 or 6 mg/kg/day) and previously tested in the noise-induced hearing loss study were presented with a 100 dB noise stimulus. If they did not exhibit clonus in response to the 100 dB noise, they were exposed to a 105 dB stimulus 24–48 h later. This was followed by an 110 dB stimulus 24–48 h later if they did not exhibit clonus at 105 dB. Female and male rats exposed to either 3 or 6 mg/kg PCBs exhibited a significantly higher incidence of audiogenic seizures, shorter latency to onset of seizures, and greater severity of seizures compared to controls. Thyroxine measured in littermates at weaning was significantly lower in all PCB groups compared to controls, suggesting a potential mechanism for the increased incidence of audiogenic seizures. This is the first study to show that developmental PCB exposure increases the susceptibility to audiogenic seizures in adulthood.

1. Introduction

Polychlorinated biphenyls (PCBs) are industrial contaminants that were manufactured for use as dielectric fluids in transformers and capacitors (Crinnion, 2011). Their production has been banned since the late 1970s, but due to their chemical stability and lipophilicity, they continue to persist in the environment. PCBs have bio-accumulated and bio-magnified up the food chain, and human exposure is primarily via consumption of contaminated fish and seafood (Crinnion, 2011). PCBs readily cross the mammalian placenta and are mobilized from body fat into breast milk during lactation, putting the developing offspring at risk (Jacobson et al., 1984). One cause for concern is that developmental exposure to PCBs has been associated with long-lasting hearing deficits in animal models and humans (Crofton et al., 2000; Goldey et al., 1995; Jusko et al., 2014; Poon et al., 2011; Powers et al., 2006; Trnovec et al., 2010).

In an early study, maternal exposure to Aroclor 1254 (a commercial PCB mixture) led to increased auditory thresholds at low frequencies in the offspring (Goldey et al., 1995). Later, Crofton et al. (2000) reported loss of outer hair cells in the region of the cochlea responsible for low-frequency hearing in adult rats after perinatal exposure to Aroclor 1254. These findings led to additional experiments to further elucidate the cochlear site of action of PCBs using distortion product otoacoustic emissions (DPOAEs), which test the integrity of the outer hair cells, and auditory brainstem responses, which measure the integrity of the auditory neuronal pathway. Exposure to Aroclor 1254 or to an environmentally relevant PCB mixture (Fox River PCB Mix; Kostyniak et al., 2005) during development resulted in decreased DPOAE amplitudes that lasted into adulthood in the absence of any changes in amplitudes

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or latencies of auditory brainstem responses, further suggesting that PCBs act at a cochlear site to produce auditory deficits (Lasky et al., 2002; Powers et al., 2006, 2009).

Given the evidence that PCBs act at the level of cochlea outer hair cells, we investigated the interaction between PCB exposure during cochlear development and exposure to loud noise in adulthood (Poon et al. unpublished results). We hypothesized that early PCB exposure might increase the likelihood of noise-induced hearing loss later in life. Unexpectedly, when exposed to loud noise, many rats in the highest PCB dose group (6 mg/kg PCB) exhibited bouts of wild running followed by a period when they were unresponsive to stimuli. These behaviors appeared to be similar those that occur during audiogenic seizures (reviewed in Ross and Coleman, 2000), and these preliminary results led to the current study to investigate this more directly.

Audiogenic seizures are elicited by exposure to loud noise, require the activation of the auditory brainstem and are initiated mainly in the inferior colliculus (Coleman et al., 1999; Eells et al., 2004; Faingold, 2002; Ishida et al., 1995; Ross and Coleman, 2000). These seizures present differently from forebrain induced seizures which start with facial and forelimb tremors and progress to a clonic or clonic-tonic state (Racine, 1972). Interestingly, the Long-Evans rats used in our studies are generally less susceptible to seizures than other rat strains, but can be made more susceptible to audiogenic seizures by priming with damaging noise during the critical period of cochlear development (Ross and Coleman, 1999, 2000).

One hypothesis for this induced sensitivity to audiogenic seizures is that priming to loud noise during cochlear development results in short-term hearing loss during this critical developmental window, resulting in a permanent down regulation of inhibitory circuits in the inferior colliculus. This serves to amplify the weaker afferent signals coming from the cochlea. This imbalance toward excitatory signaling leads to a hypersensitivity to intense stimulation later in life (reviewed in Ross and Coleman, 2000). If hearing loss can increase the sensitivity to audiogenic seizures, then PCB-induced hair cell damage and the resultant hearing deficits (Poon et al. unpublished results) may underlie the apparent increase in seizure responsiveness we observed.

In this study, our goal was to directly investigate whether developmental exposure to the Fox River PCB mixture would make rats more susceptible to audiogenic seizures in adulthood. We used a classic audiogenic seizure testing paradigm in which the rats were exposed to a brief high intensity noise (Ross and Coleman, 1999) and the incidence, severity and latency to onset of seizure behaviors were recorded.

2. Methods

2.1. Animals

Primiparous female Long–Evans rats, approximately 8–10 weeks of age, were purchased from Harlan (Indianapolis, IN) in three cohorts. They were individually housed in standard polycarbonate plastic shoebox cages with corn-cob bedding, and fed rat chow (Harlan Teklad rodent diet (W) 8604) and water ad libitum. All rats were housed in a temperature- and humidity-controlled room (22 °C, 40–55% humidity) on a 12/12 h light cycle (lights on at 0830). Average sound pressure levels in the housing room were less than 55 dB, a level well below that which would be expected to lead to hearing loss. In addition, all animals were housed in the same room, and thus were exposed to the same daily noise exposure.

The rats were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the National Institutes of Health (2002) and National Research Council (2003).

2.2. Exposure

Female rats were randomly assigned to exposure groups and treated daily with one of four dosing solutions consisting of corn oil vehicle or 1, 3, or 6 mg/kg PCBs in corn oil. Each exposure group was represented in each of the three cohorts. Exposure began 28 days prior to breeding and continued until weaning of the pups on postnatal day (PND) 21. The PCB mixture (Fox River PCB mixture) was formulated to mimic the congener profile found in walleye, a popular sport-caught fish from the Fox River in northeast WI. The mixture consisted of 35% Aroclor 1242 (Monsanto lot KB 05-415; St. Louis, MO), 35% Aroclor 1248 (AccuStandards lot F-110; New Haven, CT), 15% Aroclor 1254 (Monsanto lot KB 05-612), and 15% Aroclor 1260 (AccuStandards lot 021-020) (Kostyniak et al., 2005). The doses of the PCB mixture were selected based on the results of earlier studies assessing the in vivo developmental toxicity and auditory toxicity of this mixture in rats (see Kostyniak et al., 2005; Powers et al., 2006). The PCBs diluted in corn oil (Mazola) or the corn oil vehicle was pipetted onto one-half of a vanilla wafer cookie (Keebler Golden Vanilla Wafers) at a volume of 0.4 mL solution/kg body weight. The individual dosing solutions were mixed at concentrations of 2.25 mg/mL, 7.5 mg/mL and 15 mg/mL for the PCB doses of 1 mg/kg, 3 mg/kg and 6 mg/kg respectively. Females were weighed and fed the PCB or vehicle-treated cookies daily.

2.3. Breeding, pregnancy, and weaning

After the initial four weeks of PCB exposure, each female was paired with an unexposed male Long-Evans rat (Harlan, Indianapolis, IN) in a hanging wire cage for 8 consecutive days with food and water ad libitum. Females were returned to their home cages briefly each day for PCB dosing. Females were monitored daily for the presence of a sperm plug in order to establish gestational day 0.

On the day of parturition (PND 0), pups were examined for abnormalities, sexed and weighed. On PND 2, litters were culled to 10 pups (five males and five females when possible), and litters with at least 7 pups had extra pups cross-fostered into them from the same treatment group to bring the litters to 8–10 pups. Cross-fostered pups were ear-notched and not used for the experiment. There were 42 of 60 successful litters. Of the remaining dams, 9 were not pregnant and 9 had litters too small to be included in the study ($\leq$7 pups). Overall, non-pregnant dams and dams with small litters were evenly distributed across the treatment groups.

Dosing of the dams continued until the pups were weaned on PND 21. Although the dams were not separated from the pups during the postnatal dosing, the researchers directly gave the cookie to the dam and the cookies were consumed by the dams in a few minutes without the offspring receiving any portion. One male and one female per litter were retained for a noise-induced hearing loss study beginning at approximately PND 200 (Poon et al. unpublished results) and the audiogenic seizure testing reported here was conducted in these same animals at approximately PND 400.

All animals were subjected to some noise during habituation. Habituation consisted of exposing the rats to noise increasing in intensity from 75 to 97 dB over a period of 15 min. This occurred every day prior to exposure to 97 dB of broadband noise centered around 8 KHz for 4 h a day for 5 consecutive days. Based on previous research, this degree of noise exposure was expected to