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Determining the Critical Window of Influence of PCB Perinatally on Behavioral and Hormonal Development in Sprague-Dawley Rat Pups

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PCB Effects on Behavioral and Hormonal Development in Sprague-Dawley Rat Pups

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DETERMINING THE CRITICAL WINDOW OF INFLUENCE OF PCB PERINATALLY ON BEHAVIORAL AND HORMONAL DEVELOPMENT IN SPRAGUE-DAWLEY RAT PUPS. Natalie Sommerville, nsommer@bgsu.edu; Mikayla Bond,mikaylb@bgsu.edu; Bailey Guerin, guerinb@bgsu.edu; Lee Meserve, lmeserv@bgsu.edu; H. Casey Cromwell, hcc@bgsu.edu; Biology and Psychology Departments, Bowling Green State University, Bowling Green, Ohio 43403.

Abstract

Polychlorinated biphenyls (PCB) had widespread use in the United States in commercial manufacturing in the United States until the late 1970s. Even though they were banned, measurable amounts can still be found in the environment and food sources. PCB has known effects on altering hormone development and behavior in the species *Rattus norvegicus*. To determine the most crucial developmental time of exposure to PCB in Sprague-Dawley rat pups, rat pups were exposed to PCB at differing weeks of either gestation period or the first postnatal week. Behavioral tests were performed for the different rat pups, as well as blood serum was analyzed for the concentration of thyroid hormone. This paper presents data for PCB exposed pups that were tested for play behavior on PND 22. Statistical tests were used to determine the week of PCB exposure with the greatest effects on the rat pups. The results are that exposure of PCB led to a significant increase in the play behavior of the pups. The dorsal contact play behavior was significantly altered by PCB, while the pin behavior was not significantly altered. The critical window for PCB exposure was not identified, but it can be seen that PCB alters dorsal contact behavior in exposed rat pups.

Introduction

PCB had a number of applications in manufacturing like electrical capacitors, transformers, plastics, flame retardant liquids, hydraulic fluids and sealants. These compounds continue to bio-accumulate in the environment because of their stable structure, resulting in a long half-life and high lipophilicity. PCB can be found widely in the tissues of animals, such as fish, and can be ingested. PCB can collect in adipose tissues and can lead to potentially harmful health effects over long-term exposure. Previous studies have shown that PCB exposure can alter social behavior and the present work extends this research. PCB molecules can be classified based on the position of the chlorine atom(s) and the congeners can alter physiological processes like brain function, growth, and endocrine and reproductive development (Dover et al., 2014).

When PCB is added to the rat chow of Sprague-Dawley rats during pregnancy, it alters the thyroid status of the mother and the offspring, as well as the behavior of the dams and the pups. PCB exposure can pass from mother to offspring through the placenta during gestation and the milk during nursing (Dover et al., 2014). These effects have been found in previous studies to cause possible linkages to hypothyroidism and altered neurological development, and learning deficits similar to those with autism or attention deficit hyperactivity disorder (Johansen et al., 2013). PCB exposure at moderate doses can lead to diminished communication abilities and alteration in social behavior, which are major symptoms of autistic spectrum disorder (Krishnan et al., 2014).

This study was designed to determine whether the effects on rat pup behavior are correlated with the time of PCB exposure during pregnancy or lactation of the female rats. This study evaluated how two rat pups from the same litter, after being separated overnight, would interact with each other when they are placed back into contact with one another. Rat pups begin to play with each other actively when their eyes open (at around 2 weeks). Some of the play activity includes chasing, pinning, and wrestling with each other. When rats increase in age past four weeks, behavior decreases. The play behavior in this study was video recorded for ten minutes and then was scored manually offline. Video recordings were used to prevent alteration of normal play behavior if being watched by the investigator.

Materials and Methods

In this experiment, female Sprague-Dawley rats were used that weighed between 200-259 g and were paired with males of the same strain. Females were determined to be pregnant by a sperm positive vaginal smear. Once pregnant, the dams were fed either 25 ppm PCB diet or standard rat chow, to act as the control diet, according to their assigned group. The pregnant dams were placed into one of five groups, one of controls and four of one-week windows for PCB exposure during development (Figure 1). The weight of the dam was recorded and food consumption was monitored throughout their gestation period and the first week after pups were born. The PCB 25 ppm diet was a combination of 25 g of stock PCB 47, 50 g of stock PCB 77, and 925 g standard rat chow mash resulting in 25 mg/kg PCB of chow (25 ppm). The day that the rat pups were born was noted as PND 0. Ultrasonic vocalizations (USVs) were recorded on postnatal day (PND) 3, 7, 14, 21, and 22. Then behavioral tests were observed in the pups: grooming (PND 14), open field test (PND 21), and play behaviors (PND 22). Thyroid hormone analysis was collected from blood serum on PND 3, 7, 14, 21, and 22 (Figure 2). This particular study focuses on the play behavior that was observed on PND 22 for the rat pups.

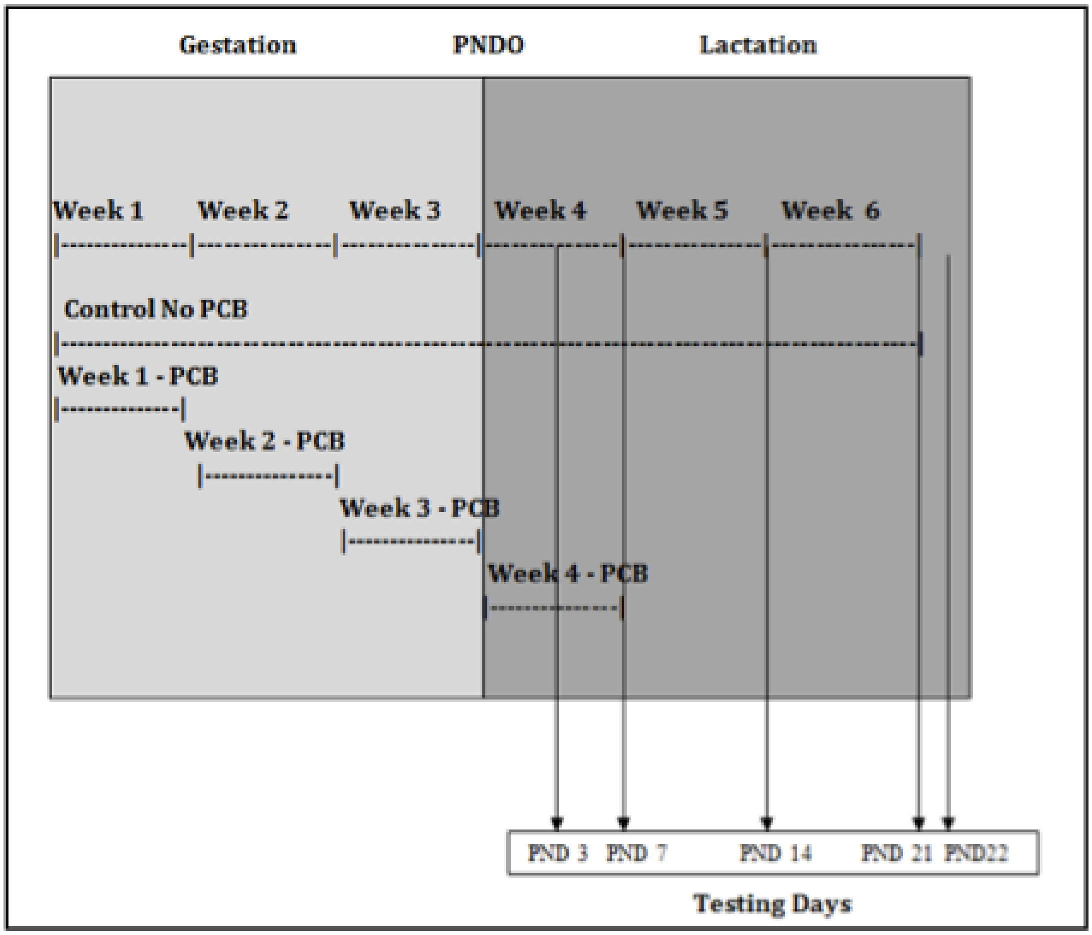

Figure 1, Experimental Design Pregnant mother rats were assigned to either a control group or four different weeks of PCB exposure for a one-week developmental "window."

Pup Landmark Days

PND 0: Pups first detected with mother

PND 3: Litter size to 8 pups (4M/4F): USV record on excess

PND 7: USV on 2 pups (1M/1F)

PND 14: Grooming and USV on 2 pups (1M/1F)

PND 21: Open Field and USV on 2 pups (1M/1F)

PND 22: Play and USV on 2 pups (1M/1F)

Figure 2, Testing Schedule on Pups Outline of the behavioral tests, USVs, and Blood Serum test dates. Play Behavior was performed on PND 22.

Results

There was significant impact of PCB on the number of dorsal contacts in the 22-day-old rat pups. The results revealed that rats exposed to PCB during gestational week 2, (P = 0.02), gestational week 3 (P = <0.03), and postnatal week (P = 0.01) all experienced a significant increase in dorsal contacts compared to the control group (Table 1). These data suggest that animals exposed to PCB have an alteration in behavior that makes them more playful in dorsal contacts. However, there was no significant effect of PCB on the number of pins regardless of when PCB was administered. This could be because the length of PCB exposure was insufficient to alter behavior (Figure 3). Exposure of pregnant rats to PCB at this level during gestational week 1 resulted in termination of the pregnancy, so there were no play behavior data collected for this developmental time.

	Dorsal Contacts	Significance v. Controls	Pins	Significance v. Controls
Control	25.8 ± 7.4		2.6 ± 1.9	
PCB Exposed				
GW 1	109	0.01	0	n.s.
GW 2	74 ± 16.0	0.02	0.5 ± 0.5	n.s.
GW3	58.7 ± 4.8	0.03	0	n.s.
PW1	104.2 ± 28.1	0.01	3.4 ± 2.3	n.s.

Table 1, Play behavior on Postnatal Day 22 Means represent number of dorsal contacts and pins during a 10 minute play period.
*n.s.=not significant, the p-value was not less than 0.5
*GW= Gestational week
*PW= Postnatal week

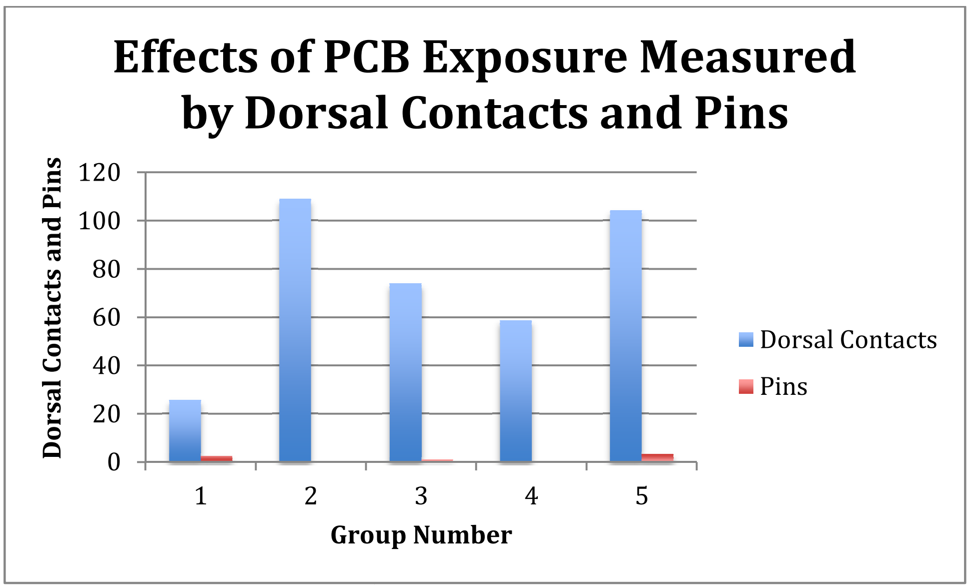

Figure 3, Image of Effects of PCB Exposure Measuring the Play Behavior The outcome of the pin behavior was not altered significantly by PCB in this study. The first group are the controls, while 2-5 are GW1-3 and PW1.

Discussion

The results of this study reveal that PCB exposure for one week during development can cause significant changes in play behavior between Sprague-Dawley rat pups. Dorsal contact play behavior was significantly altered by PCB among the rat pups, where the pin behavior was not significantly modified, regardless of time of PCB exposure. The present study differs from previous work to discover if there is a critical window of development that PCB exposure leads to the greatest amount of play behavior. Previous studies have worked with two-week windows for PCB influences on behavior in Sprague-Dawley rat pups, but the results were not significant (Baldwin et. al, 2014). The results of this study did not find one week that was significantly greater than the rest, but the greatest number of dorsal contacts came from gestational week 1 (GW1) with an average of 109 pins. Overall, the attempt to identify a critical one-week window for PCB exposure did not result in clear finding as was hypothesized. If the rats were used solely for this behavioral study maybe the results would differ instead of performing multiple behavioral tests to these rats. In conclusion, the data provide additional support to the findings that PCB changes behavior. However, it appears that this particular measure (dorsal contacts) is altered regardless of the time of PCB exposure.

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