SEX-DEPENDENT BEHAVIORAL CHANGES IN RAT OFFSPRING AFTER IN UTERO ADMINISTRATION OF A SINGLE LOW DOSE PBDE 47

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Introduction

Increasing levels of polybrominated diphenyl ethers (PBDEs) in environmental and human samples has resulted in intensive discussion regarding possible hazard identification and risk assessment in the last years. In rodents, exposure to PBDE mixtures or single congeners has resulted in a mixed induction of CYP450-dependent enzymes, showing increased activity of hepatic EROD and PROD [1;2]. In addition, genotoxicity has been observed in recombination assays [3], and neurotoxicity has been reported in mice exposed during development [4-6;6]. Acute and sub-chronic exposures of mice and rats to a PBDE mixture (DE-71) cause dose-dependent reductions in serum concentrations of thyroxin (T4), and stress-induced elevations in plasma corticosterone [1;7]. Further, some hydroxylated metabolites of PBDE congeners exhibit a higher potency *in vivo* than T4 in competitive binding to human transthyretin (TTR)[8], the transport protein mediating transfer of thyroid hormones across the placenta and into the brain. The available information in the literature clearly indicates that PBDEs are potent neurotoxicants, causing effects at doses lower than that able to disrupt thyroid hormone profiles and change CYP 450 activities. Neurobehavior effects, which includes defects in learning and memory, and changes in nicotinic receptors were found at doses starting at 0.45 ppm in mouse [9]. The congeners, PBDE 47 and PBDE 99, have also been shown to cause permanent aberrations in spontaneous behavior in mice which was more pronounced with increasing age [5].

PBDE 47 is the most predominant congener found in environmental and human samples, including human breast milk [10;11]. Its presence in breast milk highlights the importance of evaluating possible effects following early developmental

exposure and because this period represents a critical time which an organism is extremely susceptible to minor changes in hormonal milieu. Variances in terms of time point and concentration of exposure to steroids can lead to an organizational change which could manifest itself in an irreversible fashion at later time points in life.

We administered a single dose to gravid dams on gestation day 6 of either 140 µg/kg BW or 700 µg/kg BW of the congener, 2,2'4,4'-tetrabromo diphenyl ether (PBDE 47). These doses are pertinent to human exposure levels because a study by She *et al.* found a mean level of 33.3 µg PBDE 47 /kg fat in human breast adipose tissue with a range from 7.01 to 196 µg PBDE 47 /kg fat [(10)]. In this study, peri-pubertal behavior effects were evaluated in rat offspring after *in utero* administration of low dose PBDE 47.

Materials and Methods

Animals and treatment: Wistar dams (N control=17; PTU=17; PBDE 140=22 and PBDE 700=18) were treated by gavage on gestation day 6 with a single dose of 140 or 700 µg PBDE 47/kg body weight or peanut oil (control). An additional group was administered the goitrogen, PTU (6-n-propyl-2-thiouracil), which served as a reference control. PTU was given to the gravid dams by placing 5mg/L PTU in the drinking water on gestation days 7 through postnatal day 21. **Open Field:** Evaluation of exploratory activity behavior was tested on postnatal day (PND) 80 using 15 males and 15 females per group (from 15 litters). The open field consists of a circular arena (1 m diameter) enclosed by white walls (height, 40cm), which is divided into 19 equal sectors with a subdivision of central and peripheral circles. It was illuminated by a white-60 W bulb placed 80 cm above the center and the behavior of individual animal was recorded, using a DVD-camcorder (Sony, DCR-DVD 101). Rats were filmed for 5 minutes between 9:00 and 11:00 a.m. by the same investigator and between every animal session, the arena was washed with ethanol solution (10%) to avoid possible odor bias. Then number of 1- sectors crossed (locomotion), 2- rearings, 3- fecal pellets, 4- time spent self-grooming and 5- time spent in the central area was scored by the same observer. **Locomotor activity:** Basal locomotor activity was measured over a 24 h period in an automated device (Mobilitron). This device has three infra-red photocells per cage which monitor the motility of individual animals in 5 min interval, being possible to test 48 animals simultaneously. On PNDs 35 and 70, the motility of one male and one female per litter was monitored during a 24 h period after two hours habituation period. Data are expressed as a ratio of total light beam interruptions (LBI) and duration of activity per day. **Statistical analysis:** Data are expressed as the median and groups were tested by Kruskal-Wallis followed by Mann-Whitney U-test. Differences were considered statistically significant when $P \leq 0.05$.

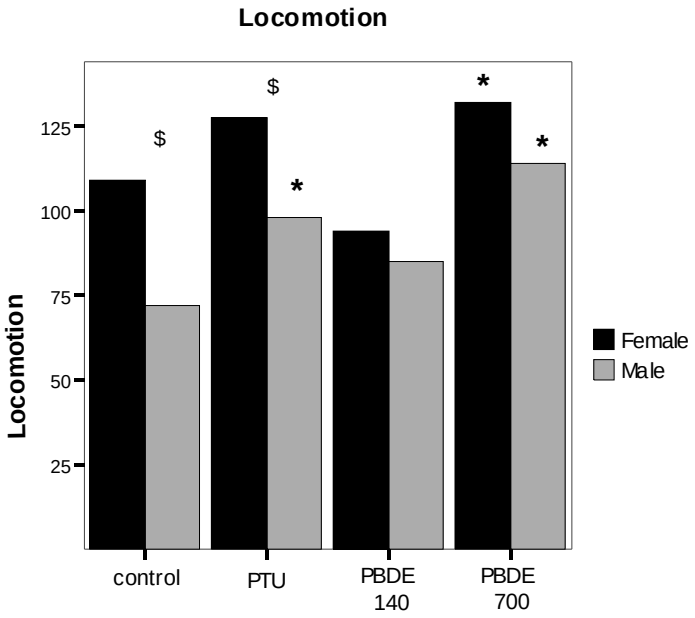


Figure 1: Locomotion of rat offspring in the open field test (PND 80). Animals were tested for 5 min and total locomotion is expressed as the total number of sectors crossed in the round arena. Values are expressed as median and significance were detected by Mann-Whitney *U*-test when $p \leq 0.05$.

$ statistically significant difference between male and female in the same

Table 1: Exploratory and emotional behavior related to open field test in rats exposed in utero *to low dose PBDE 47.*
Values are represented by median, Q1 and Q3 and differences were evaluated using the Mann-Whitney *U*-test and statistical significance was achived when $p \leq 0.05$

		Control		***PTU***		***PBDE 140***		***PBDE 700***	
		Female	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*
Rearing (n)	*Q1*	*19*	*16*	*23*	*15*	*21*	*13*	*25*	*23*
	Median	***27***	***21***	***32***	***23*** $^{\$}$	***28***	***21***	***29***	***26***
	Q3	*32*	*30*	*43*	*29*	*33*	*29*	*39*	*28*
Grooming time (s)	*Q1*	*6*	*4*	*0*	*0*	*0*	*0*	*0*	*0*
	Median	***10***	***9***	***0 ****	***6***	***3 ****	***0***	***0 ****	***8***
	Q3	*16*	*11*	*16*	*13*	*9*	*12*	*8*	*10*
Time in the central area (s)	*Q1*	*12*	*10*	*14*	*17*	*18*	*19*	*22*	*22*
	Median	***19***	***21***	***20***	***24***	***27***	***23***	***33 ****	***31***
	Q3	*31*	*43*	*32*	*31*	*34*	*33*	*42*	*50*
Fecal pellets (n)	*Q1*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
	Median	***0***	***4*** $^{\$}$	***0***	***2***	***0***	***3***	***0***	***1***
	Q3	*2*	*7*	*2*	*5*	*3*	*7*	*2*	*5*

$ statistically significant difference compared to females
* statistically significant difference compared to control

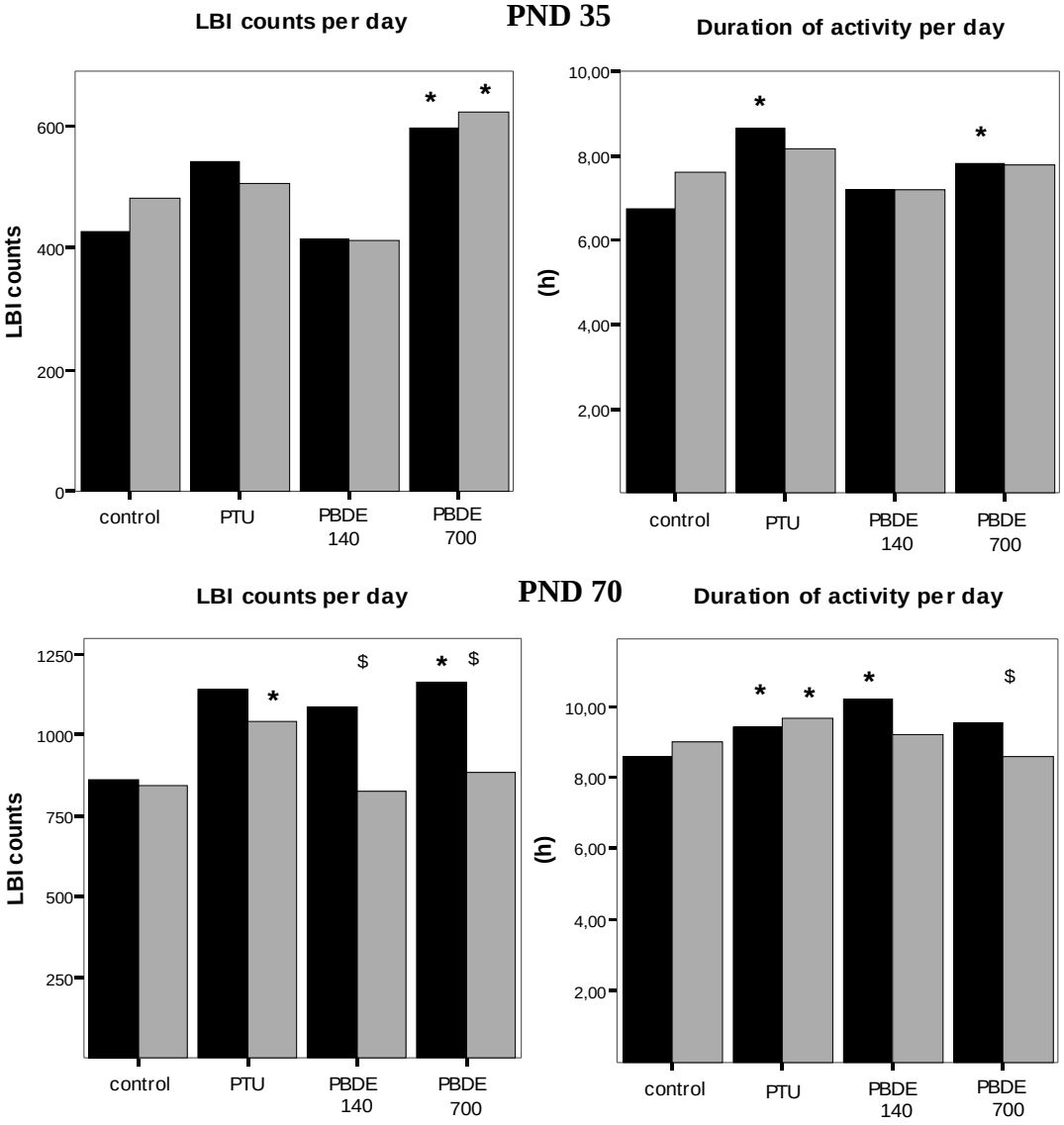
Results and Discussion

The exposure to a low dose of PBDE 47 during gestation / lactation (via milk) produced neurobehavioral changes in offspring that were apparent at least until PND 80. Using the Mobilitron apparatus, which allows us to perform 24-hour measurement of the spontaneous locomotor activity ("basal activity") of individual rats, we observed that both males and females from the PBDE 700 group were hyperactive on PND 35 (Figure 2). However, examination on PND 70 showed that the effect persisted only in females with males displaying activities comparable to control (Figure 2). On PND 70, the PTU treated offspring exhibited significantly higher LBI counts and duration of activity per day. Furthermore, in the PBDE 700

group the exploratory activity (the total number of sectors crossed) assessed in the open field test was also increased compared to controls (Figure 1). Our data is supported by Eriksson et al. (2001) who also reported that neonatal exposure (single dose on postnatal day 10) to PBDE 99 or 47 disrupts spontaneous behavior in mice and increases activity levels in a dose-response fashion which appear to be permanent and become more pronounced with age [5]. Our data confirm the findings reported by Mead *et al.* in untreated rats who showed that the "basal" activity level did not differ between males and females under baseline conditions. [12] We observed gender differences on PND 70 in the group exposed to 700µg PBDE 47, with females being more active than males.

In order to investigate the sex-dependent effects associated with low dose PBDE 47 exposure, exploratory locomotor activity was evaluated in an open field test. The open field test is one of the most widely used tests in behavioural research which allows investigation of sex-dependent differences and emotional measurements. When rats are exposed to an unfamiliar environment, females are more active than males [12;13]. In our experiment, control females moved significantly (number of sectors crossed) more than males. This effect was abolished when animals were exposed to PBDE 47 which might suggest a sex-dependent effect (Figure 1). Previously, it has been shown that adult male offspring exposed to PCBs and PBDE 99 exhibit decreased circulating steroid levels and increased saccharin intake (sweet preference test), suggesting feminization of behaviour [14]. It is well known that during development sex steroids have important organizational effects that cause persistent structural and functional differentiation on the developing brain. In the open field test, previous studies have shown that sexual dimorphism was abolished in rats when they were exposed to testosterone or estradiol during the perinatal period [15;16]. Therefore, one can not rule out the possibility that PBDE 47 might cause hormonal disturbances during critical periods of development which could modulate the observed behavioural sex-difference in the offspring. However, further investigation should be done in order to characterize such an effect.

Figure 2: "Basal" locomotor activity of rat offspring during 24-hour period in the Mobilitron machine. Animals were tested on PNDs 35 and 70 and total locomotion was expressed as total light beams count (LBI) and duration of activity per day. Values are expressed as the median and significance were detected by Mann-Whitney *U*-test when $p \leq 0.05$.



$ statistically significant difference between male and female in the same group

* statistically significant different from control

The open field test also allows reliable measurements of emotions. Fear response of an animal exposed to a novel environment is characterized by a high defecation rate, low ambulation score and less time spent in the central part of the arena [17]. Grooming has been interpreted as a displacement activity or a way of releasing the tension caused by a stressful situation. For example, although self-grooming does not seem to have any specificity for antidepressant action of drugs, in animals treated acutely with sulpiride or raclopride the grooming behaviour appears to decrease [19]8. In this study, females treated either with PBDE 47 or PTU had a significant reduction in the grooming time (not seen in males) (Table 1). In addition, females from the PBDE 700 group tended to spend more time in the central area. These changes suggest decreased fear and emotionality in the females exposed to PBDE 47 because animals displaying fear would spend less time in the central area and grooming is considered to be a possible indication of stress reactivity or emotionality by some authors (cited in *(18)9*).

We can draw three conclusions regarding the neurobehavior disturbances caused by pre- and postnatal (via milk) exposure to low dose PBDE 47 in rats: 1- our data corroborate the reported evidence of hyperactivity-induced by PBDEs in rodents; 2 – we demonstrate that PBDE 47 caused behavioural sex-dependent differences in offspring; 3 – our data also suggest that in the open field test, females exposed to a low dose PBDE 47 appear to have decreased emotionality. We observed neurobehavior changes at doses pertinent to human exposure levels indicating that the neurotoxic risks of the PBDE 47 congener should be explored further.

Acknowledgements

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