Journal of Scientific Research
Banaras Hindu University, Varanasi

Vol. 58, 2014 : 59-73
ISSN : 0447-9483

SEX-SELECTIVE ALTERATIONS IN OXIDATIVE STATUS AND SPATIAL MEMORY PERFORMANCES IN MOUSE FOLLOWING EXPOSURE OF PBDE-209 DURING BRAIN GROWTH SPURT PERIOD

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Abstract

Exposure of decabromodiphenyl ether (PBDE-209) during early brain development interferes in cognitive development. However, sex-selective effects of PBDE-209 on the brain oxidative status and subsequent spatial memory performances are still far from clear. We have therefore, investigated the alterations in the levels of malondialdehyde (MDA), protein carbonyl (PC) and in the activities of the antioxidant enzymes e.g. superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) in the frontal cortex (FC) and hippocampus (Hc) of male and female mice at postnatal day (PND) 11 and 60 after exposure of PBDE-209 at the doses of 6 or 20 mg/kg body weight during postnatal day (PND) 3-10. The spatial memory of male and female young mice (PND 60) was also evaluated by Morris water maze (MWM) and radial arm maze (RAM). Postnatal exposure of PBDE-209 significantly increased the MDA and PC levels, while decreased the activities of SOD and GSH-Px in FC and Hc of male and female mice at PND 11. After exposure, the young males followed similar pattern as the neonates, interestingly, young females did not show significant changes in MDA, PC levels and SOD, GSH-Px activities. Moreover, postnatal PBDE-209 exposure caused a significant decrease in the percent correct choices whereas, increase in working and reference memory errors in the young males only. The present study, therefore, suggests that postnatal exposure of PBDE-209 affects the oxidative status and spatial memory performances in sex-selective manner.

Key words : Brain development, Brain growth spurt period, Decabromodiphenyl ether, Oxidative stress, Spatial memory

Introduction

Polybromininated diphenyl ethers (PBDEs) are used as flame-retardant additives in many polymer products (BSEF 2000). Various recent studies have reported the ubiquitous presence of lower (penta- and octa-) and higher brominated (deca-) PBDEs in the environment, animals and humans (Domingo, 2004). Due to growing environmental and human health concerns such as nervous system damage, endocrine disruption and cancer, the production of lower brominated PBDEs is banned in the developed countries (Cox et al., 2003), however, still being used in developing countries (Tai et al., 2011). On the other hand, higher brominated PBDEs (e.g. PBDE-209) are still being used in developed as well as developing countries (Yang et al., 2011) except few developed countries (USEPA, 2010) because it is commonly

considered that higher brominated PBDEs (e.g. Decabromodiphenyl ether, PBDE-209) are less toxic and less bioaccumulative than those of lower brominated PBDEs. However, recent study has reported that PBDE-209 can bioaccumulate in the environment, biomagnify up the food chain (Bartrons et al., 2012) and exhibits toxicity similar to the lower PBDEs (Viberg et al., 2003, 2007). PBDE-209 is extensively metabolized and debrominated down to lower brominated congeners in animal bodies, so the potential toxicity of PBDE-209 may be higher than that of the lower PBDEs (Van den Steen et al., 2007). Furthermore, recent reports indicate the presence of higher level of PBDE-209 in infants and toddlers than adults (Toms et al., 2009). Therefore, PBDE-209-induced effects must be properly evaluated for their potential toxicity especially during postnatal period.

Brain is one of the most affected organ by bioaccumulant toxins due to its high lipid content and high energy requirements (Giordano et al., 2008), especially during postnatal period in mice and third trimester in humans, called brain growth spurt period (Davison and Dobbing, 1968). During this period, rapid growth and various remarkable changes occur in the brain. Neuronal oxidative damage might be one of the primary mechanism of neurotoxicity caused by PBDEs (Zhang et al., 2010; Daubié et al., 2011). It has been demonstrated that PBDEs intoxication during postnatal period persistently affects the memory in young ones (Viberg et al., 2006, 2007). Developmental PBDE exposure causes oxidative stress by altering the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Chen et al., 2010) and can affect learning and memory functions (Yan et al., 2012) which appear to worsen with age (Viberg et al., 2003). Brain produces high level of reactive oxygen species (ROS) and operates efficient antioxidant enzyme systems to counteract with the deleterious effects of oxidative stress (Magistretti, 2003). Further, male and female exhibit differential response with oxidative stress due to their qualitative and quantitative differences during development of the brain (Massafra et al., 2002). Therefore, the aim of present study is to explore whether (1) PBDE-209 sex-selectively modulates the oxidants-antioxidants homeostasis in the brain of neonates, (2) such modulations are long-term and persistent up to young-age and (3) such modulations in oxidative status is aligned with the spatial memory behavior during young-age in both the sexes.

Materials and Methods

Chemicals

PBDE-209 (98%, CAS no. 1163-19-5) was purchased from Aldrich-Chemie while corn oil from Sigma (St. Louis, MO, USA). All other chemicals were obtained from Aldrich-Chemie, Sigma, Merck and Sisco Research Laboratory (India).

Animals and PBDE-209 treatment

Male and female adult Swiss albino mice weighing 25–30 g were maintained in an animal house as per the recommendations from Central Animal Ethical

Committee of the University (CAECU) for the care and use of laboratory animals. The animals were maintained at ambient temperature and 12L/12D cycle. They were fed with standardized pelleted food and tap water *ad libitum*. Two females were housed with one male for breeding. Females were examined every morning to observe the formation of vaginal plug. The vaginal plug-positive females were caged individually. The day of litter born was designated postnatal day (PND) 0. The size of the litter was adjusted as much as possible in order to obtain litters of the same size (6-8 pups) and even distribution of male and female pups within each litter. Male and female pups were not separated at birth and thus each litter contained both sexes. At PND 0, male and female pups within the same litter were randomly assigned to different treatment groups. 84 pups of each sex at PND 3 were used in the present study. The pups were divided into 3 groups having 28 males and 28 females in each. Three days old pups in Group I were administered with corn oil which served as vehicle-treated control while pups of Group II and III were administered with 6 and 20 mg/kg body weight (bw) of PBDE-209, respectively (Viberg et al., 2007). All the treatments were given orally via a micropipette with 100 µl microtip at a volume of 5 µl/gm bw of pups from PND 3-10. Pups of each group were divided into two subgroups I and II, comprising of 7 and 21 pups of each sex, respectively. Seven pups of each sex from both subgroups were sacrificed on PND 11 and 60 for biochemical analyses. Seven pups of each sex of subgroup II were used for Morris water maze (MWM) test from PND 60-66 while in rest of the seven pups of each sex, radial arm maze (RAM) test was conducted from PND 60-74. FC and Hc were dissected out and stored at -80°C for biochemical analysis. FC and Hc regions of the brain were chosen as these they are involved in learning and spatial memory functions (Davachi et al., 2003).

Biochemical Analyses

Homogenates of FC and Hc were prepared in 50 mM phosphate buffer (pH 7.0) containing 1.0 mM phenylmethanesulfonylfluoride and centrifuged at 10,000×g for 10 minutes at 4°C. Protein contents of supernatants were measured by Bradford method with minor modifications (Gupta and Kanungo, 2013; Gupta and Prasad, 2013). The levels of malondialdehyde (MDA) and protein carbonyl (PC) were measured by biochemical assay whereas the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were detected by in-gel activity assay as described in detail previously (Verma et al., 2013).

Learning and memory behavioural tests

Modified Morris water maze (MWM) and radial arm maze (RAM) have been used to evaluate the memory deficit caused by PBDE-209.

Morris water maze

MWM, a black-painted circular water tank (diameter: 122 cm, height: 51 cm), was divided into four equal quadrants – Q1, Q2, Q3 and Q4. The tank was filled with water up to the height of 31 cm. A square platform (area: 10 cm^2, height: 30 cm),

placed in the center of one of these four quadrants was typically submerged 1.0 cm below the water surface filled. Platform was kept in the target quadrant Q2 (South-West) throughout the training session. Acquisition trials (working memory) and probe trials (reference memory) were performed by MWM as described in our previous report (Verma et al., 2013).

Radial arm maze

The RAM consisted of a round central platform (40 cm) elevated 50 cm above the floor, with eight radiating 32 cm long and 5 cm wide arms. Each arm forms a corridor leading to a square platform (8 cm^2) having a small cup of 1 cm in diameter containing a hidden reward. The correct choices, working and reference memory errors were tested by RAM as described previously (Verma et al., 2013).

Statistical Analysis

Data were presented as means ± standard errors of means (SEM). The biochemical estimations and in gel activity assay were evaluated with three-way analysis of variance (ANOVA) between subject factors sex, age and treatment. In MWM, acquisition trial was analyzed by three-way ANOVA between subject factors sex, session and treatment, whereas probe trial was analyzed by one-way ANOVA. The radial arm maze data were also analyzed by using three-way ANOVA between subject factors sex, session block and treatment. All the analyses were followed by post hoc Tukey HSD (honestly significant difference) test. All statistical analyses were conducted using SPSS (16.0) software. A difference of $p<0.05$ was considered statistically significant for main effects, however, for interactions at $p<0.1$.

Results

Sex-selective effect of PBDE-209 on the lipid peroxidation

For level of MDA in FC, three-way ANOVA indicated the significant main effects of sex ($F_{1,72}$ 101.103, p=0.000), age ($F_{1,72}$ 203.416, p=0.000) and treatment ($F_{2,72}$ 173.798, p=0.000) and the significant effect of sex x age ($F_{1,72}$ 14.696, p=0.000), sex x treatment ($F_{2,72}$ 36.573, p=0.000) and age x treatment ($F_{2,72}$ 8.103, p=0.001) interactions (Fig. 1A). By contrast, significant effect of sex x age x treatment ($F_{2,72}$ 1.955, p=0.149) interaction was lacking. Similarly, in Hc (Fig. 1B), main effects of sex ($F_{1,72}$ 38.512, p=0.000), age ($F_{1,72}$ 99.790, p=0.000) and treatment ($F_{2,72}$ 200.300, p=0.000) and the interactions of sex x age ($F_{1,72}$ 54.232, p=0.000), sex x treatment ($F_{2,72}$ 16.139, p=0.000), age x treatment ($F_{2,72}$ 12.158, p=0.001) and sex x age x treatment ($F_{2,72}$ 19.063, p=0.000) were significant (Fig. 1B).

Sex-selective effect of PBDE-209 on the protein carbonylation

For level of PC in FC (Fig. 2A), three-way ANOVA indicated the significant main effects of sex ($F_{1,72}$ 85.138, p=0.000), age ($F_{1,72}$ 82.153, p=0.000) and treatment

($F_{2, 72}$ 431.652, p=0.000) and the significant effects of sex x treatment ($F_{2, 72}$ 6.251, p=0.003) and age x treatment ($F_{2, 72}$ 29.451, p=0.000) interactions. By contrast, significant effects of sex x age ($F_{1,72}$ 0.481, p=0.489) and sex x age x treatment ($F_{2, 72}$ 1.055, p=0.353) interactions were lacking (Fig. 2A). Similarly, in Hc, main effects of sex ($F_{1,72}$ 44.026, p=0.000), age ($F_{1, 72}$ 815.400, p=0.000) and treatment ($F_{2, 72}$ 320.683, p=0.000) and the interactions of sex x treatment ($F_{2, 72}$ 8.129, p=0.001) and age x treatment ($F_{2, 72}$ 28.887, p=0.000) were significant. However, significant effects of sex x age ($F_{1,72}$ 0.854, p=0.458) and sex x age x treatment ($F_{2, 72}$ 1.218, p=0.302) interactions were lacking (Fig. 2B).

Sex-selective effect of PBDE-209 on the activity of SOD

For activity of SOD in FC, three-way ANOVA indicated the significant main effects of sex ($F_{1,66}$ 23.212, p=0.000), age ($F_{3, 66}$ 1.227, p=0.000) and treatment ($F_{2, 66}$ 12.626, p=0.000) and the significant effect of age x treatment ($F_{6, 66}$ 72.042, p=0.000) interaction, however, significant effect of the interactions were lacking in sex x age ($F_{1,66}$ 0.006, p=0.939), sex x treatment ($F_{2, 66}$ 1.369, p=0.262) and sex x age x treatment ($F_{2, 66}$ 2.105, p=0.130) (Fig. 3A). Similarly, in Hc, significant main effects of sex ($F_{1,66}$ 298.957, p=0.000), age ($F_{3, 66}$141.288, p=0.000) and treatment ($F_{2, 72}$ 37.595, p=0.000) were noticed. The interactions of sex x age ($F_{1,66}$ 78.494, p=0.000), sex x treatment ($F_{2, 66}$ 12.094, p=0.000), age x treatment ($F_{6, 66}$ 7.501, p=0.000) and sex x age x treatment ($F_{2, 66}$ 2.105, p=0.130) were also significant (Fig. 3B).

Sex-selective effect of PBDE-209 on activity of the GSH-Px

For activity of GSH-Px in FC, three-way ANOVA indicated the significant main effects of treatment ($F_{2, 24}$ 193.566, p=0.000), whereas, significant main effects were lacking in sex ($F_{1, 24}$ 0.428, p=0.519) as well as in age ($F_{1, 24}$ 0.164, p=0.689). The interactions of sex x age ($F_{1, 24}$ 110.154, p=0.000), sex x treatment ($F_{2, 24}$ 15.052, p=0.000), age x treatment ($F_{2, 24}$ 11.518, p=0.000) and sex x age x treatment ($F_{2, 24}$ 71.134, p=0.130) were significantly apparent (Fig. 4A). Similarly, in Hc, significant main effects of age ($F_{1, 24}$ 52.800, p=0.000) and treatment($F_{2, 24}$ 77.644, p=0.000) and significant effects of sex x age ($F_{1, 24}$ 23.909, p=0.000), age x treatment ($F_{2, 24}$ 13.764, p=0.000) and sex x age x treatment ($F_{2, 24}$ 6.578, p=0.005) were observed on the activity of GSH-Px whereas, significant effects were lacking in sex ($F_{1, 24}$ 0.003, p=0.954) and sex x treatment ($F_{2, 24}$ 0.997, p=0.384) (Fig. 4B).

Sex-selective effect of PBDE-209 on Morris Water Maze performance

The escape latency time (ELT) was analyzed for successive 6 days by three-way ANOVA in male and female mice at PND 60. With respect to ELT, significant

effects were observed for sex ($F_{1, 216}$ 15.620, p=0.000), treatment ($F_{2, 216}$ 3.384, p=0.036), session ($F_{5, 216}$ 23.771, p=0.000) and interaction of sex x session ($F_{1, 216}$ 15.620, p=0.000). However, no significant effects were observed for sex x treatment ($F_{2, 216}$ 1.142, p=0.321), treatment x session ($F_{10, 216}$ 0.35, p=0.979) and sex x treatment x session ($F_{10, 216}$ 0.175, p=0.998) interactions (Fig. 5A).

During probe trial on day 7, mice spent significantly more time ($p<0.05$) in quadrant Q2 (target) relative to other quadrants (Q1, Q3 and Q4) in all the groups ($p<0.05$). In 6 and 20 mg/kg PBDE-209-exposed males, a significant decrease in the time spent in quadrant Q2 ($p<0.05$) was found as compared with their respective controls ($p<0.05$). The PBDE-209-exposed female mice spent significantly more time ($p<0.05$) in quadrant Q2 (target) relative to other quadrants (Q1, Q2 and Q3) in all the groups. PBDE-209 exposures at both doses did not decrease the time spent in the quadrant Q2 ($p<0.05$) as compared with the control (Fig. 5B).

Sex-selective effect of PBDE-209 on Radial Arm Maze performance

The % correct choices, reference and working memory errors were analyzed for successive 12 days. This was presented as 2-days session blocks over 12 days of training for male and female of control and both the treated groups.

With respect to % correct choices, ANOVA indicated main significant effects of session block ($F_{5, 216}$ 44.168, p=0.000) as well as treatment ($F_{2, 216}$ 14.398, p=0.000), whereas, significant effect of sex ($F_{2, 216}$ 1.792, p=0.182) was not observed. Significant effects were observed for sex x session block ($F_{5, 216}$ 1.866, p=0.101), sex x treatment ($F_{2, 216}$ 2.611, p=0.076) and session block x treatment ($F_{10, 216}$ 3.755, p=0.182) interactions. However, no significant effect of sex x session block x treatment ($F_{10, 216}$ 0.999, p=0.445) interaction was found (Fig. 6A).

With respect to reference memory error, three way ANOVA indicated main significant effects of session block ($F_{5, 216}$ 13.939, p=0.000) as well as treatment ($F_{2, 216}$ 7.107, p=0.001), whereas, significant effect of sex ($F_{2, 216}$ 1.021, p=0.313) was not observed. Significant effects were observed for sex x session block ($F_{5, 216}$ 2.005, p=0.071) and session block x treatment ($F_{10, 216}$ 1.781, p=0.065) interactions. However, no significant effects of sex x treatment ($F_{2, 216}$ 0.266, p=0.766) and sex x session block x treatment ($F_{10, 216}$ 0.999, p=0.445) interactions were found (Fig. 6B).

With respect to working memory error, three ANOVA indicated main significant effects of sex ($F_{1, 216}$ 64.737, p=0.000), session block ($F_{5, 216}$ 10.595, p=0.000) and treatment ($F_{2, 216}$ 7.248, p=0.001). Significant effects were also observed for sex x session block ($F_{5, 216}$ 22.894, p=0.000) and sex x treatment ($F_{2, 216}$ 3.329,

p=0.038) interactions. However, no significant effects of session block x treatment ($F_{10, 216}$ 1.174, p=0.310) and sex x session block x treatment ($F_{10, 216}$ 1.498, p=0.141) interactions were found (Fig. 6C).

Discussion

PBDE-209 exposure during the brain spurt period, results in neurotoxicity (Tagliaferri et al., 2010) and subsequent behavioural impairments and memory deficits. Since, sex and age are speculated as very important factors in contributing the oxidant/antioxidant balance, we investigated the effects of PBDE-209 exposure on the oxidative status in FC and Hc of mice of both sexes at PND 11 and 60 as well as spatial memory test in the later.

Our findings suggest that exposure of PBDE-209 during postnatal period (PND 3-10) results in significant increase in the levels of MDA and PC while significant decrease in the activities of SOD and GSH-Px in the FC and Hc at PND 11 of both the sexes. Our result is partially consistent with the findings of Chen et al. (2010) who reported that *in vitro* exposure of PBDE-209 affects secondary messengers, oxidative stress and global gene DNA methylation levels in primary cultured hippocampal neurons of neonatal rat at PND 1. Our results indicate that neonates of both sexes showed more responsiveness towards the alterations in the oxidative stress following postnatal exposure of PBDE-209. This might be due to low activities of antioxidant enzymes and reduced ability of antioxidant defenses resulting into declined free radical–trapping capacity (Tsukahara et al., 2004). SOD is considered to be the first line of defence against deleterious effects of ROS (Linares et al., 2009). The decreased level of SOD observed in FC and Hc following postnatal exposure of PBDE-209 in both sexes of mice at PND 11 indicates the generation of large number of free radicals which is evidenced by increase in lipid peroxidation and protein carbonylation also. Moreover, reduction in GSH-Px activity might reflect an inability of these regions of the brain to eliminate hydrogen peroxide produced by exposure of PBDE-209 in these mice. These changes can cause disturbances in ROS/antioxidants homeostasis during the rapid growth period of the postnatal mice brain when a series of fundamental developmental changes occur like neurogenesis, axonal outgrowth, dendritic sprouting, establishment of neural connections, synaptogenesis, vascularisation and proliferation of glia cells with accompanying myelinisation (Rice and Barone, 2000). It is also worth to mention that during development, when animals acquire many new sensory and motor abilities (Bolles and Woods, 1964) as well as the peaks of spontaneous motor behavioural activity (Campbell et al., 1969), increased burden of ROS and perturbed levels of antioxidant enzymes activity may induce developmental neurotoxicity which may affect brain development, growth and maturation. Interestingly, our results

indicate recovery in ROS/antioxidant enzyme imbalance in young females who were exposed to PBDE-209 postnatally whereas the males failed to show such restorations. This might be due to presence of optimal levels of sex steroids such as estrogen and progesterone in young females which are reported to be involved in neuroprotection (Ozacmak and Sayan, 2009). The neuroprotective capability of estrogen is due to its antioxidant effects, interaction with membrane binding sites and modulation of neurotransmitter systems (Badeau et al., 2005). These factors might have resulted into smaller persistent alterations in the young females than the males who are more prone to the long-term alterations following postnatal exposure of PBDE-209. It is reported that both 17β-estradiol and its isomers are involved in neuroprotection against oxidative stress in various experimental models in vitro (Biewenga et al., 2005). According to Badeau et al. (2005) the antioxidant activity of steroid is dependent on the presence of a hydroxyl group on the C3 position of the A ring, which is independent of the activation of estrogen receptors and hence can be correlated with neuroprotection provided by estrogens. In present study, there may be the possibility of fluctutations in the level of estrogen from postnatal period till the attainment of puberty resulting in the differential responses of female neonates and young ones towards oxidative stress found in various regions of the brain.

In our study the sex-selective effects of postnatal PBDE-209 exposure on the ROS/antioxidant enzymes balance appear to be related with late-emerging deficits in the behaviour of young mice. Our studies are consistent with Forster et al. (1996) and Liu et al. (2003), as they have reported a correlation between lipid peroxidation and protein carbonylation with learning and memory impairments. Fukui et al., (2002) have reported that such increased oxidative degeneration of lipids and proteins in nerve terminal membranes may change the membrane surface potential, resulting in a deterioration of the neurotransmission systems due to decline in the membrane fusion between nerve terminal membranes and synaptic vesicles, including the neurotransmitter. The synaptic vesicles accumulate abnormally in nerve terminals due to oxidative stress (Fukui et al., 2002) resulting in extensive lesions in cortex, thalamus, hypothalamus, and striatum that ultimately result into the memory impairments (Lin et al., 1995). In our study, the untreated control mice presented the normal sex difference in the learning and memory task, with the males exhibiting significantly fewer working and reference memory errors than the females. This is consistent with the previous findings indicating the better performances in spatial learning in Morris water maze (Brandeis, 1989) and an 8-armed radial maze by the male rodents (Astur et al., 2004). The present finding also shows that working memory and reference memory are significantly impaired in young males, however, no significant changes could be noticed in young females exposed with PBDE-209 postnatally. Our study is

articulated with the findings of Levin et al. (2010), who indicated the sex-selective impairment in learning and memory and unaltered level of neurotransmitter in young rats following postnatal exposure of parathion. Oxidative damage of the hippocampus and cerebral cortex in rats is thought to contribute in impairment of cognitive functions such as learning and memory deficits (Fukui et al., 2001). We also noticed significant changes in oxidative status and in spatial memory behaviour following postnatal exposure of PBDE-209 in young males while absence of such changes in females, indicating a direct correlation between oxidative stress and spatial memory behaviour. Further, based on earlier reports, the possibility of the involvement of sex-steroids in neuroprotection against PBDE-209-induced toxicity can not be ruled out in young female mice.

Thus it can be concluded that the postnatal exposure of PBDE-209 causes early-stage effect as neurotoxic insult in both sexes of mice whereas neurochemical as well as neurobehavioral impairments persisted for a long-term, only in the males. The present study also indicates a marked correlation between spatial memory and cellular markers of brain oxidative stress in young males and females. Whether the oxidative stress-induced changes in the learning and memory caused by postnatal exposure of PBDE-209 persist till old age or not, need to be explored.

Acknowledgement

PV is thankful to BHU for providing Junior Research Fellowship and to Indian Council of Medical Research, Govt. of India, for Senior Research Fellowship (Award No. 45/56/2011/TRM-BMS). We thank to Late Padma Shri Prof. Madhu S Kanungo and Dr. Rajaneesh K Gupta, Department of Zoology, BHU, India for extending laboratory facilities and extensive discussion on the work respectively.

References

Astur RS, Tropp J, Sava S, Constable RT, Markus EJ (2004) Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation. *Behav Brain Res* 151: 103-115.

Badeau M, Adlercreutz H, Kaihovaara P, Tikkanen MJ (2005) Estrogen A-ring structure and antioxidative effect on lipoproteins. *J Steroid Biochem Mol Biol* 96: 271–278.

Bartrons M, Grimalt JO, de Mendoza G, Catalan J. Pollutant Dehalogenation Capability May Depend on the Trophic Evolutionary History of the Organism: PBDEs in Freshwater Food Webs. *PLOS ONE 2012* 7(7): e41829. doi:10.1371/journal.pone.0041829.

Biewenga E, Cabell L, Audesirk T (2005) Estradiol and raloxifene protect cultured SN4741 neurons against oxidative stress. *Neurosci Lett* 373: 179–183.

Bolles RC, Woods PJ (1964) The ontogeny of behavior in the albino rat. *Animal Behav* 12: 427-441.

Brandeis R, Brandys Y, Yehuda S (1989) The use of the Morris water maze in the study of memory and learning. *International J Neurosci* 48: 29-69.

Bromine Science and Environment Forum (BSEF) (2000) An introduction to brominated flame retardants. BSEF, Belgium.

Campbell BA, Lytle LD, Fibiger HC (1969) Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* 166: 635-637.

Chen J, Liufu C, Sun W, Sun X, Chen D (2010) Assessment of the neurotoxic mechanisms of decabromodiphenyl ether (PBDE-209) in primary cultured postnatal rat hippocampal neurons includes alterations in second messenger signaling and oxidative stress. *Toxicol Letters* 192: 431-439.

Chen JR, Yan YT, Wang TJ, Chen LJ, Wang YJ, Tseng GF (2009) Gonadal Hormones Modulate the Dendritic Spine Densities of Primary Cortical Pyramidal Neurons in Adult Female Rat. *Cereb Cortex* 1-9.

Cox P, Ethymiou P (2003) Directive 2003/11/EC of the European parliament and of the council of February 6, 2003 amending for the 24th time Council Directive 76/669/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). *Official J the European Union OJ L* 42: 45-46.

Cull-Candy S, Brickley S, Farrant M (2001) NMDA receptor subunits: diversity, development and disease. *Curr Opin Neurobiol* 11: 327–335.

Daubié S, Bisson JF, Lalonde R, Schroeder H, Rychen G (2011) Neurobehavioral and physiological effects of low doses of polybrominated diphenyl ether (PBDE)-99 in male adult rats. *Toxicol Letters* 204: 57-63.

Davachi L, Mitchell JP, Wagner AD (2003) Multiple routes to memory: distinct medial temporal lobe processes build item and source memories. *Proc of the Natl Acad Sci of USA* 100: 2157-2162.

Davison AN, Dobbing J (1968) *Applied neurochemistry*. Oxford: Blackwell, pp.178–221.

Domingo JL (2004) Human exposure to polybrominated diphenyl ethers through the diet. *J Chromat A* 1054: 321-326.

Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS (1996) Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc of the Natl Acad Sci of USA* 93: 4765-4769.

Fukui K, Omoi N, Hayaska T, Shinnkai T, Suzuki S, Abe K, et al. (2002) Cognitive impairment of rats caused by oxidative stress and aging, and its prevention by vitamin E. *Ann NY Acad Sci* 959: 275-284.

Fukui K, Onodera K, Shinkai T, Suzuki S, Urano S (2001) Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. *Ann NY Acad Sci* 928: 168-175.

Giordano G, Kavanagh TJ, Costa LG (2008) Neurotoxicity of a polybrominated diphenyl ether mixture (DE-71) in mouse neurons and astrocytes is modulated by intracellular gluthatione levels. *Toxicol and Applied Pharmacol* 232: 161-168.

Gupta RK, Kanungo M (2013) Glial molecular alterations with mouse brain development and aging: up-regulation of the Kir4.1 and aquaporin-4. *Age (Dordr)* 35:59-67.

Gupta RK, Prasad S. (2013) Early down regulation of the glial Kir4.1 and GLT-1 expression in pericontusional cortex of the old male mice subjected to traumatic brain injury. *Biogerontol* 14:531-541.

Jelks KB, Wylie R, Floyd CL, McAllister AK, Wise P (2007) Estradiol Targets Synaptic Proteins to Induce Glutamatergic Synapse Formation in Cultured Hippocampal Neurons: Critical Role of Estrogen Receptor-α. *J Neurosci* 27: 6903–6913.

Levin ED, Timofeeva OA, Yang L, Petro A, Ryde IT Wrench N (2010) Early Postnatal Parathion Exposure in Rats Causes Sex-Selective Cognitive Impairment and Neurotransmitter Defects Which Emerge in Aging. *Behav Brain Res* 208: 319-327.

Lin MT, Kao TY, Jin YT, Chen CF (1995) Interleukin-1 receptor antagonist attenuates the heat stroke-induced neuronal damage by reducing the cerebral ischemia in rats. *Brain Res Bulletin* 37: 595-598.

Linares V, Alonso V, Albina ML, Bellés M, Sirvent JJ, Domingo JL, et al. (2009) Lipid peroxidation and antioxidant status in kidney and liver of rats treated with sulfasalazine. *Toxicol* 256: 152-156.

Liu R, Liu IY, Bi X, Thompson RF, Doctrow SR, Malfroy B, et al. (2003) Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. *Proc of the Natl Acad Sci of USA* 100: 8526-8531.

Magistretti PJ (2003) Brain energy metabolism. In: Fundamental neuroscience (2nd ed.), Elsevier Science, New York, pp. 339–360.

Massafra C, Gioia D, De Felice C, Muscettola M, Longini M, Buonocore G (2002) Gender related differences in erythrocyte glutathione peroxidase activity in healthy subjects. *Clinical Endocrinol (Oxford)* 57, 663-667.

Ozacmak VH, Sayan H (2009) The effects of 17β estradiol, 17α estradiol and progesterone on oxidative stress biomarkers in ovariectomized female rat brain subjected to global cerebral ischemia. *Physiol Res* 58: 909-912.

Rice D, Barone S (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animals models. *Environ Health Perspect* 108: 511–533.

Rudick CN, Woolley CS (2001) Estrogen regulates functional inhibition of hippocampal CA1 pyramidal cells in the adult female rat. *J Neurosci* 21: 6532-6543.

Tagliaferri S, Caglieri A, Goldoni M, Pinelli S, Alinovi R, Poli D, et al. (2010) Low concentrations of the brominated flame retardants BDE-47 and BDE-99 induce synergistic oxidative stress mediated neurotoxicity in human neuroblastoma cells. *Toxicol In Vitro* 24: 116-122.

Tai J, Zhang W, Che Y, Feng D (2011) Municipal solid waste source separated collection in China: a comparative analysis. *Waste Management* 31:1673–1682

Toms L, Sjödin A, Harden F, Hobson P, Jones R, Edenfield E, et al. (2009) Serum polybrominated diphenyl ether (PBDE) levels are higher in children (2-5 years of age) than in infants and adults. *Environ Health Perspect* 117: 1461-1465.

Tsukahara H, Jianga MZ, Ohtaa N, Satoa S, Tamuraa S, Hiraokaa M, et al. (2004) Oxidative stress in neonates: Evaluation using specific biomarkers. *Life Sciences* 75: 933–938.

USEPA (United States Environmental Protection Agency) An Exposure Assessment of Polybrominated Dipheyl Ethers. Washington DC: USEPA; 2010 May. pp. 378.

Verma P, Singh P, Gandhi BS (2013) Prophylactic efficacy of *Bacopa monnieri* on decabromodiphenyl ether (PBDE-209)-induced alterations in oxidative status and spatial memory in mice. *Asian J Pharm Clin Res* 6(3): 242-247.

Viberg H, Fredriksson A, Eriksson P (2007) Changes in spontaneous behavior and altered response to nicotine in the adult rat, after postnatal exposure to the brominated flame retardant, decabromodiphenyl ether (PBDE-209). *Neurotoxicology* 28: 136-142.

Viberg H, Fredriksson A, Jakobsson E, Örn U, Eriksson P (2003) Neurobehavioral derangements in adult mice receiving decabromodiphenyl ether (PBDE-209) during a defined period of postnatal brain development. *Toxicological Sciences* 76: 112-120.

Viberg H, Johansson N, Fredriksson A, Eriksson J, Marsh G, Eriksson P (2006) Postnatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. *Toxicol Sci* 92, 211-218.

Van den Steen E, Covaci A, Jaspers VL, Dauwe T, Voorspoels S, Eens M, et al. (2007) Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (Sturnus vulgaris). *Environ Pollution* 148, 648-653.

Yan T, Xiang L, Xuejun J, Chengzhi C, Youbin Q, Xuelan Y, et al. (2012) Spatial learning and memory deficit of low level polybrominated diphenyl ethers-47 in male adult rat is modulated by intracellular glutamate receptors. *The J Toxicol Sci* 37, 223-233.

Yang C, Meng XZ, Chen L, Xia S (2011) Polybrominated diphenyl ethers in sewage sludge from Shanghai, China: possible ecological risk applied to agricultural land. *Chemosphere* 85:418–423.

Zhang C, Liu F, Liu X, Chen D (2010) Protective effect of N-acetylcysteine against BDE-209-induced neurotoxicity in primary cultured postnatal rat hippocampal neurons in vitro. *International J Dev Neurosci* 28: 521-528.

Legend of Figures

Fig. 1 Effect of postnatal exposure of PBDE-209 (6 and 20 mg/kg) on lipid peroxidation in frontal cortex (A) and hippocampus (B). The units of lipid peroxidation (mean ±SEM) are expressed as nmoles malondialdehyde produced per mg protein. *$p<0.05$, control vs experimental groups.

Fig. 2 Effect of postnatal exposure of PBDE-209 (6 and 20 mg/kg) on protein carbonylation in frontal cortex (A) and hippocampus (B) respectively. The units of protein carbonylation (mean ± SEM) are expressed as nmoles protein carbonyl produced per mg protein. *$p<0.05$, control vs experimental groups.

Fig. 3 Effect of postnatal exposure of PBDE-209 (6 and 20 mg/kg) on SOD frontal cortex (A) and hippocampus (B). The gel photographs are representative of three independent SOD in-gel activity assays. The histograms are representative of integrated densitometric values (IDV) of SOD bands. Results presented as mean ± SEM from the 3 independent sets of experiments. *$p<0.05$, control vs experimental groups.

Fig. 4 Effect of postnatal exposure of PBDE-209 (6 and 20 mg/kg) on GSH-Px activity in frontal cortex (A) and hippocampus (B). The gel photographs are representative of three independent GSH-Px in-gel activity assays. The histograms are representative of

integrated densitometric values (IDV) of GSH-Px bands. Results presented as mean ± SEM from the 3 independent sets of experiments. *p<0.05, control vs experimental groups.

Fig. 5 Effect of postnatal exposure of PBDE-209 (6 and 20 mg/kg) on acquisition (A) and probe trial (B) in mice at PND 60. In acquisition trials, each value represents mean±SEM. (a) indicates significance at p<0.05 of the particular day's Escape Latency Time (ELT) i.e., ELT of days 2 to 6 vs ELT on day 1. (b) and (c) indicates p<0.05 of PBDE-209 doses vs ELT of control group for the same day. During probe trials, the histogram shows effect of PBDE-209 on retrieval of memory as compared to control male mice respectively and each value represents mean±SEM. (d) indicates p<0.05 vs time spent in other quadrants, i.e., Q1, Q3, and Q4. (e) indicates significance at p<0.05 vs the control group's time spent in the target quadrant (Q2).

Fig. 6 Effect of postnatal exposure of PBDE-209 (6 and 20 mg/kg) on percentage of correct choices (A), reference memory error (B) and working memory error (C) in mice at PND 60. Data shows mean±SEM for trial blocks of 2 days. (a) indicates significance at p<0.05 (trial on day 1 vs trial on day 2-12) and (b) indicates significance at p<0.05 vs the trial of control group for same day.

Figures

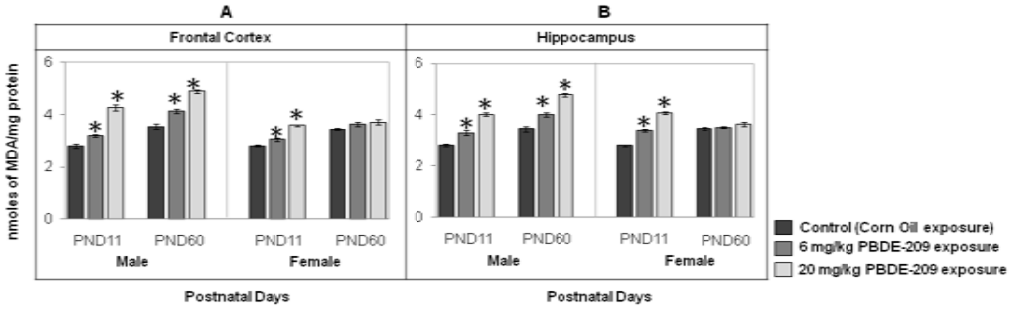

Fig. 1

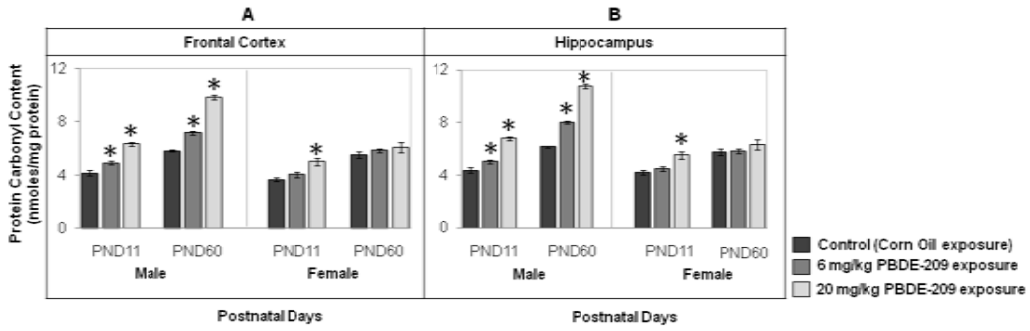

Fig. 2

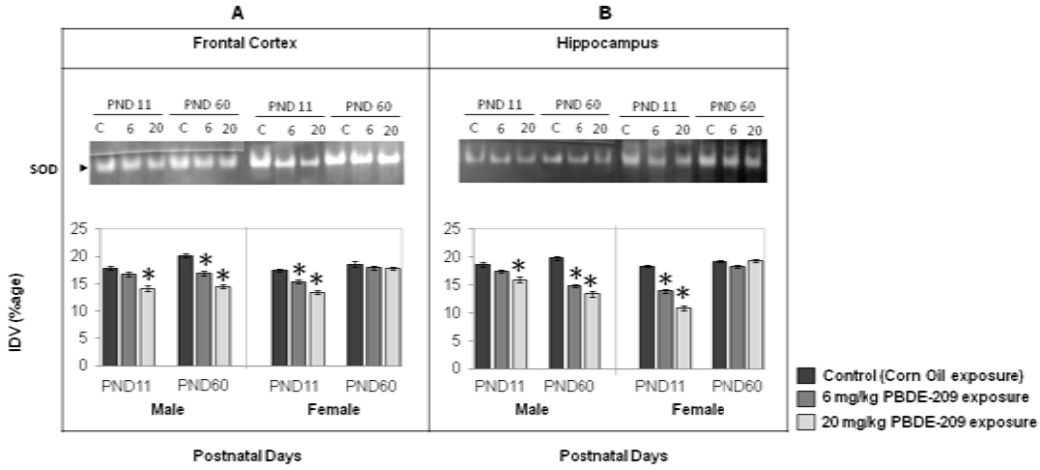
A
B
Frontal Cortex
Hippocampus
PND 11
PND 60
C 6 20
SOD
IDV (%age)
PND11
PND60
Male
Female
Postnatal Days
Control (Corn Oil exposure)
6 mg/kg PBDE-209 exposure
20 mg/kg PBDE-209 exposure

Fig. 3

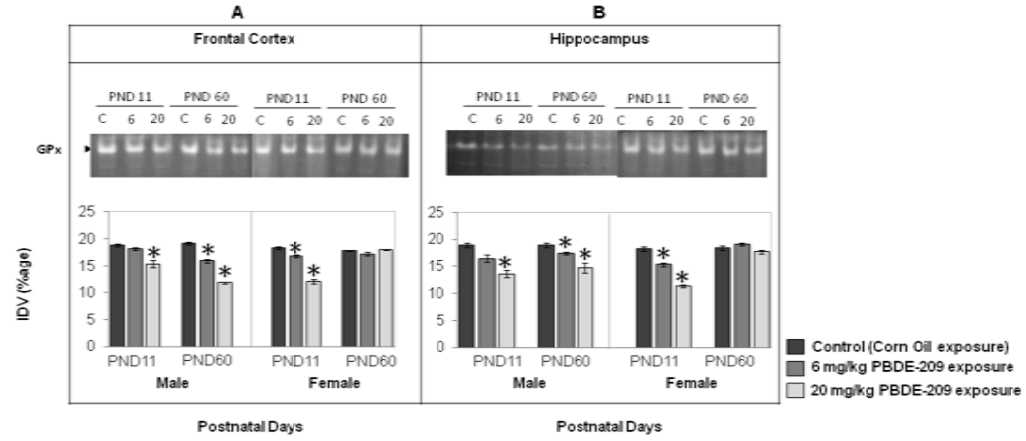
A
B
Frontal Cortex
Hippocampus
PND 11
PND 60
C 6 20
GPx
IDV (%age)
PND11
PND60
Male
Female
Postnatal Days
Control (Corn Oil exposure)
6 mg/kg PBDE-209 exposure
20 mg/kg PBDE-209 exposure

Fig. 4

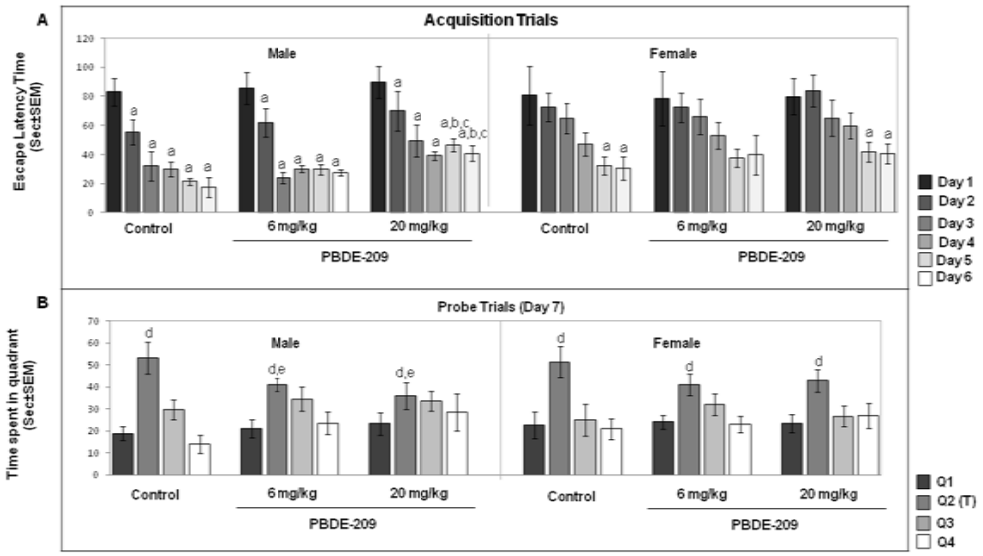
A
Acquisition Trials
Escape Latency Time (Sec±SEM)
Male
Female
Control
6 mg/kg
20 mg/kg
PBDE-209
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
B
Probe Trials (Day 7)
Time spent in quadrant (Sec±SEM)
Q1
Q2 (T)
Q3
Q4

Fig. 5

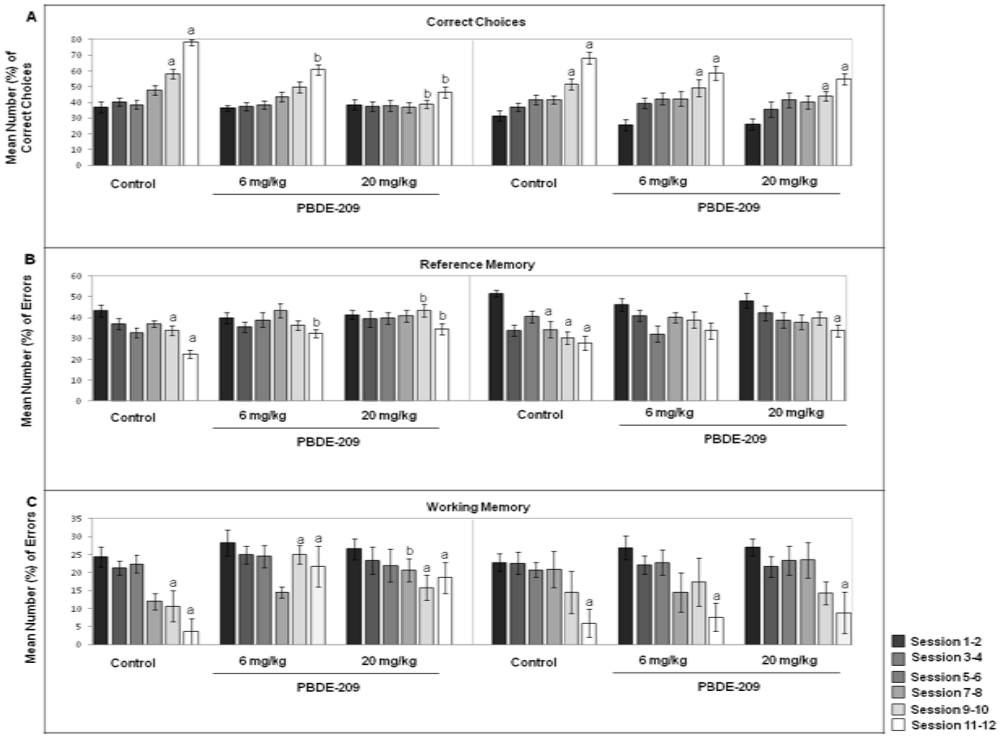
A
Correct Choices
Mean Number (%) of Correct Choices
B
Reference Memory
Mean Number (%) of Errors
C
Working Memory
Mean Number (%) of Errors
Control
6 mg/kg
20 mg/kg
PBDE-209
Session 1-2
Session 3-4
Session 5-6
Session 7-8
Session 9-10
Session 11-12

Fig. 6