

Neonatal exposure to brominated flame retardant BDE-47 changes mouse neuronal plasticity

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Introduction

Due to their widespread abundance, concern has risen about the possible neurotoxicity of brominated flame retardants (BFRs). One of the main BFRs, polybrominated diphenyl ethers (PBDEs) have been found in environmental samples, in wildlife, food, but also in human tissue and human breast milk, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) being the predominant congener (Hites 2004). Children, at the age of early brain development, accumulate BDE-47 more rapidly than adults due to their diet (breastfeeding/relatively large intake) and behavior (contact with house-dust) (Jones-Otazu et al. 2005). Distribution studies in adult and developing mice have shown that higher tissue concentrations of BDE-47 are reached in pups compared to adult mice after identical dosing regimens, presumably partly because of differences in excretion (Staskal et al. 2006).

Behavioral studies investigating effects of neonatal exposure of brominated flame retardants on learning and memory have shown adverse neurodevelopmental effects of neonatal BDE-47 exposure (Eriksson et al. 2001). The main objective of our study was to gain insight in the mechanisms underlying these previously observed neurobehavioral effects of BDE-47. To this purpose we have investigated long-term potentiation (LTP) in hippocampal slices as a measure for learning, memory and synaptic plasticity. Paired pulse facilitation (PPF), a short form of synaptic plasticity, was investigated to reveal possible presynaptic effects of BDE-47. Additionally, protein expression levels in the postsynaptic density (PSD) were investigated to further reveal underlying mechanisms. Our results demonstrate that neonatal exposure to BDE-47 results in a reduction of LTP and a reduction of a number of postsynaptic proteins involved in synaptic plasticity. These results thus provide a functional basis for previously observed neurobehavioral changes (Eriksson et al. 2001).

Methods

Male C57Bl/6 mice received a single oral dose of vehicle or 6.8 mg (14 μ mol)/kg bw BDE-47 at PND10. Recordings of field-excitatory postsynaptic potential (f-EPSP) recordings and brain protein analysis were performed at PND 17-19. BDE-47 was synthesized and purified (~99%) at the Wallenberg laboratory of

Stockholm University (Sweden). For oral dosing via a metal gastric tube, BDE-47 was dissolved in a 1:10 (w/w) mixture of egg lecithin and peanut oil.

After inhalation anesthesia (isoflurane), the animals were killed by decapitation. The brain was rapidly dissected on ice. Transverse hippocampal slices (450 μm) were cut in ice-cold carbogenated Mg^{2+} -enriched artificial cerebrospinal fluid (ACSF) using a Leica VT1000 S vibrotome. After at least 1.5 h stabilization at room temperature, f-EPSPs were recorded in the hippocampal CA1 region. Slices were superfused with carbogenated ACSF (~ 2 ml/min) in a recording chamber at 30 $^{\circ}\text{C}$. Bipolar stainless steel stimulation electrodes were placed on the afferent fibers of the stratum radiatum of the hippocampal CA1 region. f-EPSPs in CA1 were recorded with ACSF-filled glass microelectrodes using an Axoclamp-2B amplifier. Data were digitized and stored using 'Spike2' software.

During baseline recording, half-maximum f-EPSPs were evoked every 30 s. After 15 min baseline recording, LTP was induced with a single tetanic stimulation (100 Hz, 1 s) and f-EPSPs were recorded for another 30 min. PPF was recorded under identical conditions as for LTP. Interstimulus intervals of 50, 100, 200, 500 and 1000 ms were used. For data analysis, initial slopes of the f-EPSPs were determined.

For analysis of protein levels, the triton-insoluble fraction (TIF) was purified from blind samples of cortex and hippocampus of control and 6.8 mg/kg bw BDE-47-exposed animals using a previously validated biochemical fractionating method (Gardoni et al. 2006). Similar protein yield was obtained in TIF purified from cortex and hippocampi of both groups, and the same amount of protein was applied to SDS-PAGE and electroblotted for all samples. After blocking non-specific protein interactions with 10% albumin in Tris-buffered saline (TBS), the nitrocellulose papers were incubated for 2 h at room temperature with primary antibodies in 3 % albumin in TBS. After extensive rinsing in TBS/0.1 % Tween 20, the nitrocellulose papers were incubated with horseradish peroxidase-conjugated secondary antibodies. Finally, the antigen-antibody complex was revealed by enhanced chemiluminescence.

Results

Visual inspection of the brain slices of exposed pups did not show any changes of general hippocampus morphology (data not shown). The stimulus-response relation recorded in hippocampal slices was also unaffected following BDE-47 exposure (data not shown). Half-maximum f-EPSP slopes were 682 ± 138 V/s in control and 679 ± 92 V/s in BDE-47 exposed animals.

In the BDE-47-exposed mice, there was significantly less post-tetanic potentiation (PTP; 135 ± 9 %) than in the control mice (190 ± 17 %, $p < 0.01$, Figure 1A). After PTP the f-EPSP size decreases and stabilizes at a higher level than baseline, a level referred to as LTP. This level of LTP is maintained for at least 30 min. In the BDE-47-exposed mice, LTP was significantly less (130 ± 7 %) than in the control group (165 ± 16 %, $p < 0.05$, Figure 1A).

The paired-pulse ratio, determined by dividing the slope of the second average f-EPSP by the slope of the first average f-EPSP, did not differ between control and BDE-47 exposed groups (data not shown). For the 50 ms interstimulus interval the PPR amounted to 1.98 ± 0.11 % in the control group and 1.87 ± 0.15 % in

the BDE-47 group. For the 1000 ms interstimulus interval the PPR was 1.16 ± 0.03 % in the control group and 1.08 ± 0.03 % in the BDE-47 group.

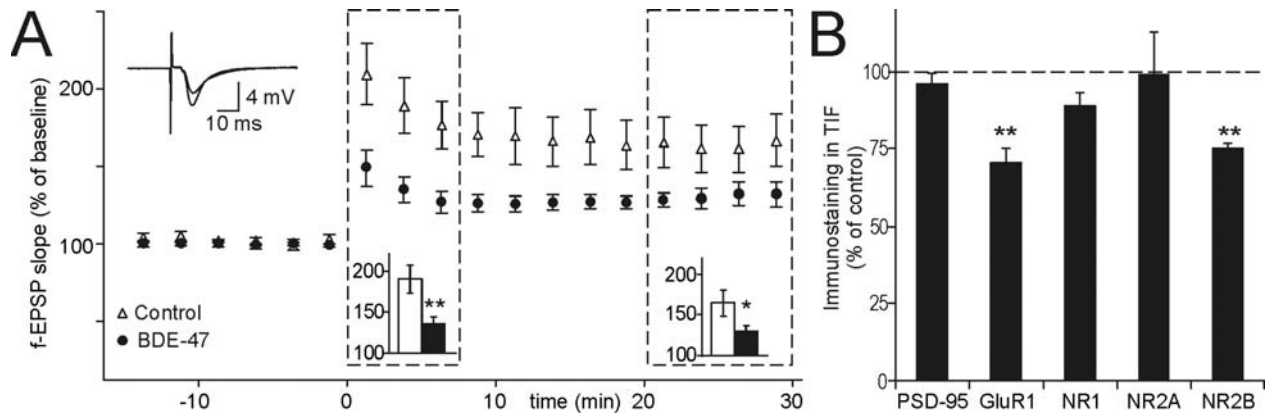


Figure 1. A. Neonatal exposure to BDE-47 reduces PTP and LTP in hippocampal neurons. PTP (0 - 7.5 min after tetanus at t = 0) and LTP (20 - 30 min) in BDE-47-exposed mice (solid circles, n=8) as compared to control mice (open triangles, n=5). The upper left inset shows superimposed traces illustrating the enhancement of f-EPSPs by LTP induction. Bar diagram insets show averages of data in the dashed frames (control: open bars, BDE-47: solid bars). **B.** Effects of BDE-47 on levels of postsynaptic protein in hippocampus. Relative amount of postsynaptic proteins in hippocampal TIF (representing the PSD) of BDE-47-exposed mice (n=4) compared to control mice (n=4). Data is presented as average \pm SEM; * $p < 0.05$; ** $p < 0.01$.

Because the NMDA receptor complex plays an important role in the development of LTP and is enriched in the post-synaptic density (PSD), we used western blot analysis to measure protein levels of NMDA receptor subunits and a PSD-associated signaling protein in total homogenate and TIF representing the PSD compartment (Gardoni et al. 2001). BDE-47 had no effects on protein levels in cortical homogenate, cortical TIF and hippocampal homogenate (data not shown). Significant changes in protein levels of NMDA receptor subunits NR1 and NR2A were not detected. However, protein levels of NMDA receptor subunit NR2B (75 ± 2 %) and α -amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) receptor subunit GluR1 (71 ± 4 %) were significantly reduced ($p < 0.01$). Protein levels of the NMDA receptor interacting protein PSD-95 were unaltered (Figure 1B).

Discussion

We report here that neonatal exposure to BDE-47 causes developmental effects consisting of a reduction of PTP and LTP, as well as a specific reduction in the levels of some postsynaptic proteins critically involved in synaptic plasticity. It is important to note that in this experimental design, the exposure to BDE-47 takes place during a period of rapid brain growth. The multitude and complexity of processes during this rapid development makes the developing brain particularly vulnerable to the effects of xenobiotics. Neurodevelopmental effects have previously been shown for a range of xenobiotics, including the adverse effect of BDE-47 on spontaneous behavior and habituation (Eriksson et al. 2001). In the present study a specific reduction of key proteins in the postsynaptic density, i.e., AMPA receptor

subunit GluR1 and NMDA receptor subunit NR2B was observed, whereas the levels of these proteins in total hippocampus homogenate were unaffected. The specific decrease in the levels of these proteins in the PSD is therefore attributed to changes in glutamate receptor subunit trafficking or clustering in the PSD instead of a reduced protein translation.

In conclusion, neonatal exposure to the environmentally abundant brominated flame retardant BDE-47 results in a reduction in long-term synaptic plasticity. Effects on quantity and nature of postsynaptic glutamate receptors, which are functionally involved in hippocampal synaptic plasticity, are also revealed. Thus the previously reported neurobehavioral effects (Eriksson et al. 2001) are now functionally supported by the demonstrated reduction of LTP and postsynaptic proteins.

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