# ONTOGENETIC ALTERATIONS IN MOLECULAR AND STRUCTURAL CORRELATES OF DENDRITIC GROWTH FOLLOWING DEVELOPMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBS)

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**Abstract**

Perinatal exposure to PCBs is associated with decreased IQ scores, impaired learning and memory, and attentional deficits in children. It is postulated that these neuropsychological deficits reflect altered patterns of neuronal connectivity. To test this hypothesis, we examined the effects of developmental PCB exposure on dendritic growth. Rat dams were gavaged from gestational day 6 through postnatal day (PND) 21 with vehicle (corn oil) or the commercial PCB mixture Aroclor 1254 (6 mg/kg/d). Golgi analyses of CA1 hippocampal pyramidal neurons and cerebellar Purkinge cells indicated that PCBs caused a pronounced age-related increase in dendritic growth. While dendritic lengths were significantly attenuated in PCB-treated animals at PND22, the rate of growth was accelerated at later ages such that by PND60, dendritic growth was comparable to or even exceeded that observed in controls. Quantitative RT-PCR analyses demonstrated that from PND4 through PND21, developmental PCB exposure generally increased expression of both spinophilin and RC3/neurogranin mRNA in the hippocampus, cerebellum and cortex with the most significant increases observed in the cortex. This study demonstrates that developmental PCB exposure alters the ontogenetic profile of dendritogenesis in critical brain regions, supporting the hypothesis that disruption of neuronal connectivity contributes to neuropsychological deficits seen in exposed children.

**Introduction**

A recent analysis of the available epidemiological data concluded that the weight of evidence indicates a negative association between developmental exposure to environmental PCB levels and measures of neuropsychological function in infancy or childhood[1]. However, the cell and molecular mechanism(s) by which PCBs derail cognitive and psychomotor development in children remain speculative. In experimental models, PCBs have been shown to alter intracellular calcium and protein kinase C signaling[2], transiently deplete dopamine levels[3], and interfere with thyroid hormone signaling[4]. How these molecular changes relate to functional deficits has been difficult to establish, in part due to the paucity of data describing effects of PCBs on specific neurodevelopmental events. One neurodevelopmental event that is influenced by intracellular calcium, dopamine and thyroid hormone signaling is dendritogenesis. Subtle perturbations of temporal or spatial aspects of dendritic growth are associated with altered behavior in experimental models, and in humans, such structural aberrations are thought to contribute to deficits observed in a variety of neurodevelopmental disorders. Hydroxylated PCB metabolites have recently been reported to inhibit thyroid hormone-dependent dendritic growth in primary cultures of mouse cerebellar Purkinje cells[5], but whether this PCBs similarly interfere with dendritic growth in vivo remains in question. The goal of this study, therefore, was to test the hypothesis that developmental PCB exposure disrupts normal ontogenetic patterns of dendritic growth in vivo.

**Materials and Methods**

Rat dams were gavaged from gestational day 6 through postnatal day (PND) 21 with vehicle (corn oil) or the commercial PCB mixture Aroclor 1254 (6 mg/kg/d). For morphometric analyses of dendritogenesis, cerebral hemispheres and cerebella were harvested from PND22 and PND60 male pups. The hippocampal formation was stained using the Rapid Golgi protocol[6] and the cerebellum was stained using a modified Golgi-Cox staining protocol[7]. Dendritic branching was quantified by Sholl analysis[8] and evaluated using the Wilcoxon rank-sign test; spine density was quantified along 30 µm terminal dendritic tip segments and evaluated by Student's T-test. Expression of spinophilin and RC3/neurogranin mRNA in the frontal cortex, hippocampus and cerebellum of male pups on PNDs 4, 7, 14, 21 and 56 were quantified by real-time RT-PCR using a Stratagene MX3000P. The amount of target transcript in experimental samples was determined by linear regression analyses using a standard curve generated independently for spinophilin and RC3/neurogranin; target gene levels were normalized against endogenous GAPDH mRNA levels. To determine statistical significance, data were log transformed to improve symmetry and stabilize variance, then analyzed by two-way analysis of variance (ANOVA) with main variables of treatment and age. If treatment effects were found to be significant ($p < 0.01$), differences between PCB-treated and control values were separately evaluated using t-tests based on the common error term from the ANOVA.

**Results and Discussion**

The PCB exposure paradigm used in these studies did not cause maternal or fetal toxicity as determined by maternal body weights during gestation and lactation, mean litter size (11.6 pups) and pup survival and weight gain. However, Golgi analyses indicated that developmental PCB exposure significantly altered dendritic growth. In CA1 hippocampal pyramidal neurons, branching in the distal two-thirds of the basilar dendritic arbor in PCB-treated animals was significantly decreased at PND22 but significantly increased at PND60 relative to age-matched vehicle controls. Comparison of the estimated cumulative length of the distal two-thirds of the basilar dendritic arbors of CA1 hippocampal pyramidal neurons as a function of age suggested that developmental PCB exposure significantly accelerated the rate of dendritic arborization between PND22 and PND60 (Figure 1). This enhanced dendritic growth did not appear to be the result of a generalized hypertrophic cellular response since the soma size of CA1 pyramidal neurons was not affected by PCB exposure at either age. Similarly, developmental PCB exposure decreased the area encompassed by the dendritic arbor of cerebellar Purkinje cells at PND22, but at PND60, the differences between the experimental and control groups was no longer evident, suggesting an accelerated rate of dendritic growth at later ages in the PCB-treated animals (Figure 2). PCB treatment had less widespread effects on spine density, with the only difference being a decrease in spine density in CA1 hippocampal neurons of PCB-treated neurons at PN22 relative to age-matched vehicle controls.

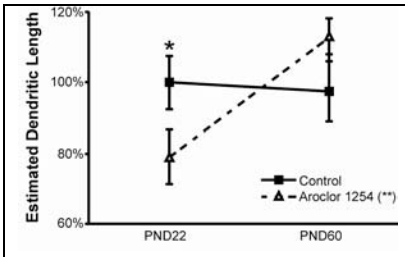


**Figure 1.** Developmental exposure to PCBs alters dendritic morphogenesis in CA1 pyramidal neurons. The estimated cumulative length of the distal two-thirds of the basilar trees as a function of age is expressed as a % of the control value at PND22. Relative to age-matched controls, the CA1 pyramidal neurons in rats exposed developmentally to Aroclor 1254 (6 mg/kg in the maternal diet) exhibit 20% less dendritic arbor at PN22 ($t = 2.136$, $p < 0.05$). At later ages, dendritic growth was accelerated in PCB treated animals such that by PND60, there was no statistically significant difference between PCB-treated and vehicle control animals.

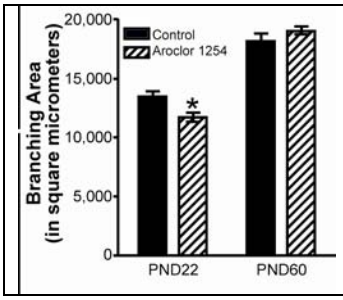


**Figure 2**. Developmental exposure to PCBs accelerates the rate of dendritic growth in cerebellar Purkinje cells between PND22 and PND60 as determined by quantitative assessment of the branching area of Purkinje cells in vehicle control and Aroclor 1254-treated rat pups as a function of age. Data are expressed as the mean ± SEM (N = 5 for control and N = 6 for PCB treated rats at PND22; N = 5 for control and N = 7 for PCB treated at PND 60; 8 neurons analyzed per cerebellum). *Significantly different from age-matched control (t = 3.013, $p < 0.005$).

The effects of developmental PCB exposure on dendritogenesis were also assessed using real-time RT-PCR to quantify expression levels of spinophilin, an actin-binding protein used as a marker of spine density, and RC3/neurogranin, a brain-specific protein kinase C substrate used as a molecular indicator of dendritic arborization. Interestingly, expression of RC3/neurogranin mRNA has previously been demonstrated to be modulated by developmental PCB exposure[9]. Developmental exposure to Aroclor 1254 generally increased both spinophilin and RC3/neurogranin levels. With respect to RC3/neurogranin, two-way ANOVA using PCB treatment and age as main effects identified no significant differences in the hippocampus between control and PCB-treated animals. Single treatment effects were observed in the cerebellum [$F(1,36) = 4.48$, $p < 0.04$]. A significant interaction between age and treatment was detected in the cortex [$F(4,38) = 4.98$, $p < 0.003$] with significant treatment differences at 4, 7 and 14 days (adjusted 2-sided $p < 0.04$). Interestingly, PCB treatment decreased RC3/neurogranin levels at PND4, but increased levels at PND7 and 14. With respect to spinophilin transcript levels, in the hippocampus there was a significant effect due to age [$F(4,38) = 65$; $p < 0.001$], but there were no significant effects due to treatment either as an interaction with age or as a main effect. In the cerebellum, an interaction between age and treatment was identified [$F(4,39) = 4.10$, $p < 0.007$) with the only significant difference indicated at PND4 (adjusted 2-sided $p < 0.0004$). A significant interaction between age and treatment was also indicated for spinophilin levels in the cortex [$F(4,37) = 4.64$, $p < 0.004$). PCB treatment significantly decreased spinophilin transcript levels at PND4 (adjusted 2-sided $p < 0.05$), and significantly increased spinophilin mRNA levels at PND7, PND14 and PND21 (adjusted 2-sided $p < 0.05$).

Our findings support the hypothesis that PCBs perturb neuronal connectivity in the developing brain by interfering with dendritogenesis. Developmental PCB exposure caused an apparent delay in dendritic growth in hippocampal CA1 pyramidal neurons at weaning as evidenced by a significant decrease in branching complexity of the distal basilar dendritic trees at PND22. However, this initial impairment of dendritic growth was followed by subsequent enhanced dendritic growth, allowing for a mature dendritic aborization that was increased relative to controls. The response seen in cerebellar Purkinje cells was similar, although less robust. Developmental PCB exposure also caused region-specific changes in the ontogenetic profile of transcripts encoding the dendrite-specific proteins spinophilin and RC3/neurogranin. Based on our molecular observations we would predict developmental PCB exposure to increase dendritic growth and/or spine formation. While our RT-PCR analyses were not predictive of the structural outcome in the hippocampus or cerebellum at weaning, they were consistent with the normal dendritic growth that occurs with maturation and support the suggestion of enhanced dendritic growth between PND22 and PND60. These data also suggest that the cortex may be more sensitive to effects of PCBs on dendritic growth than either the hippocampus or cerebellum. Since cortical functioning is critically important in learning and memory, Golgi analyses of cortical cell morphology in PCB-treated animals are warranted. Given the influence of dendritic morphology on neuronal function and the role of dendritic plasticity in learning and memory, the dysmorphic pattern of dendritic development and maturation observed in these neuronal cell populations following developmental PCB exposure could be an important factor in the cognitive impairment observed following developmental PCB exposure.

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