# CHANGES IN THE DNA-BINDING OF SEVERAL TRANSCRIPTION FACTORS IN THE DEVELOPING RAT CEREBELLUM BY PCBs

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## Introduction

PCBs are a class of persistent halogenated aromatic hydrocarbon chemical pollutants and considered as one of the major environmental contaminants resulting from intensive industrial use and inadequate disposal. In utero exposure to PCBs has been known to cause delayed neuronal development, cognitive deficits and motor dysfunction[1-2]. The cellular and molecular basis for PCB-induced developmental neurotoxicity is still unclear, however, a series of *in vitro* and some *in vivo* studies have revealed that the disruption of $Ca^{2+}$ homeostasis and $Ca^{2+}$ mediated signal transduction play a significant role[3-4]. Since the culminating event in a variety of signal transduction pathways is the regulation of gene expression[5], we examined the DNA-binding of several transcription factors such as specificity protein (Sp1), activator protein (AP-1), nuclear factor kappa-B (NFκB) and cAMP-responsive element modulator (CREB) in order to identify those that are involved in signal transduction/transcription coupling in the developing brains of PCB-exposed animals.

## Materials and Methods

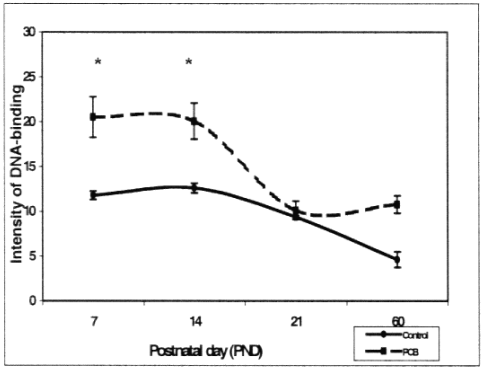
*Animal Exposure*

Timed-pregnant Log-Evans hooded rats were obtained from Charles River Laboratories (Raleigh, NC) and exposed perinatally to 0 or 6 mg/kg/day of Aroclor 1254 (Accu Standard Inc., Lot # 124-191) from gestation day 6 through postnatal day (PND) 21. All procedures were approved by the Institutional Animal Care and Use Committee of National Health and Environmental Effects Research Laboratory of the U.S. EPA. On PND 7, 14, 21, and 60, pups were removed from each litter and various brain regions were dissected and stored at -70 °C. The cerebellum was used for the present investigation.

*Nuclear Extracts preparation and Gel Mobility Shift Assay (GMSA)*

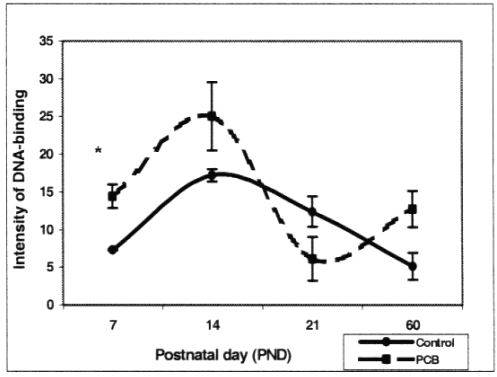
Nuclear extracts from cerebellar tissue was prepared according to the modified method of Dignam *et al*, (1983)[6]. The DNA-binding of transcription factors were monitored using GMSA[7]. The Sp1/AP-1/ NFkB/CREB oligonucleotide (Promega) was labeled with [$\gamma$-$^{32}$P] ATP (3000Ci/mmol). One µL of above labeled probe and 5-10 µg of protein from cerebellar nuclear extract were incubated in a reaction mixture containing 20 µL of gel shift binding buffer at room temperature for 10 min. The final reaction mixture along with 1µL of loading buffer was loaded onto a 6 % polyacrylamide gel and electrophoretically resolved. The gel was exposed to X-ray film overnight and the resulting autoradiograms were analyzed for shifted bands and quantitated by image acquisition and analysis software (UVP, CA).

Fig. 1



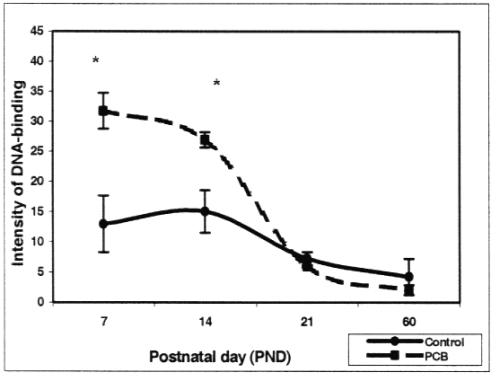

Changes in the developmental profiles of Sp1 DNA-binding in the rat cerebellum after exposure to PCBs. Sp1 DNA-binding was monitored using gel mobility shift assay. Shifted bands were scanned and quantified (see methods section). Values are means of three independent experiments.

Fig. 2



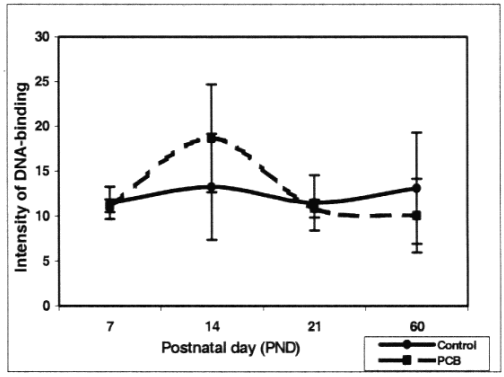

Changes in the developmental profiles of AP1 DNA-binding in the rat cerebellum after exposure to PCBs. AP1 DNA-binding was monitored using gel mobility shift assay. Shifted bands were scanned and quantified (see methods section). Values are means of three independent experiments.

Fig. 3




Changes in the developmental profiles of NFkB DNA-binding in the rat cerebellum after exposure to PCBs. NF-kB DNA-binding was monitored using gel mobility shift assay. Shifted bands were scanned and quantified (see methods section). Values are means of three independent experiments.

Fig. 4




Changes in the developmental profiles Of CREB DNA-binding in the rat cerebellum after exposure to PCBs. CREB DNA-binding was monitored using gel mobility shift assay. Shifted bands were scanned and quantified (see methods section). Values are means of three independent experiments.

**Results and discussion**

The DNA-binding of all the transcription factors studied following exposure to PCBs were initially elevated and followed by a gradual decline. Sp1 DNA-binding was increased significantly on all time points except PND 21 (Fig. 1). The developmental profile of AP-1 DNA-binding exhibited a pattern similar to Sp1 DNA-binding but the induction was significant only on PND 7 (Fig. 2). NFκB DNA-binding was dramatically elevated during PND 7 and 14 and then retuned to normal levels (Fig. 3). The changes observed in DNA-binding to CREB was minor and not significant at any time point (Fig. 4). These findings suggest that PCBs may alter transcriptional regulation machinery *in vivo* which is

characterized by a pronounced effect early after birth. The major changes observed in Sp1 and NFêB suggested that the transcriptional regulation might be mediated by both Sp1 and NFκB. The zinc finger protein (ZFP) transcription factor Sp1 is a versatile protein involved in the regulation of different genes and sensitive to various toxicants during development[8-9]. It has been proposed that an increase in the second messenger system leads to the activation of latent transcription factors[10]. Hence an increase in NFêB DNA-binding may be attributed to PCB-induced perturbations of second messenger systems. Further more, results from this study suggest that the effects of PCBs on secondary messenger systems were coupled to the gene expression.

**Acknowledgments**

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