

Imprinting of Cerebral and Hepatic Cytochrome P450s in Rat Offsprings Exposed Prenatally to Low Doses of Cypermethrin

Anshuman Singh · Sanjay Yadav · Vikas Srivastava ·
Rakesh Kumar · Dharendra Singh ·
Rao Sethumadhavan · Devendra Parmar

Received: 1 December 2012 / Accepted: 29 January 2013 / Published online: 28 February 2013
© Springer Science+Business Media New York 2013

Abstract Oral administration of low doses (1.25 or 2.5 or 5 mg/kg) corresponding to 1/200th or 1/100th or 1/50th of LD50 of cypermethrin, a synthetic type II pyrethroid, to pregnant Wistar rats from gestation day 5 to 21 produced a dose-dependent increase in the expression of xenobiotic metabolizing cytochrome P450 (CYP) 1A-, 2B- and 2E1 in the brain and liver of offsprings postnatally at 3 weeks that persisted up to 12 weeks. This persistent increase in CYPs was associated with alterations in circulating concentrations of testosterone, luteinizing hormone and follicle stimulating hormone, spontaneous locomotor activity and accumulation of cypermethrin in the brain of exposed

offsprings. Rechallenge of exposed offsprings at adulthood (12 weeks old) with cypermethrin (p.o., 10 mg/kg × 6 days) led to a much higher increase in the expression of CYPs in the exposed offsprings when compared to the control offsprings treated with cypermethrin. Further, bioinformatic analysis demonstrating absence of specific short interspersed elements in CYPs suggests that persistence in the increase in CYPs in exposed offsprings could be attributed to the imprinting of the cerebral and hepatic CYPs following prenatal exposure to low doses of cypermethrin. This imprinting could be of toxicological relevance as it may modify the response of drugs or environmental exposures in exposed offsprings particularly for those chemicals which require CYP-mediated metabolism to produce their beneficial or toxic effects.

A. Singh · S. Yadav · V. Srivastava · D. Parmar (✉)
Developmental Toxicology Division, Indian Institute of
Toxicology Research (IITR), CSIR, PO Box 80, M.G. Marg,
Lucknow 226 001 Uttar Pradesh, India
e-mail: parmardevendra@hotmail.com

A. Singh
e-mail: anshuman321_80@hotmail.com

S. Yadav
e-mail: sanjayitrc@gmail.com

V. Srivastava
e-mail: vikas@iitr.res.in

R. Kumar
Analytical Chemistry Division, CSIR-IITR, Lucknow,
Uttar Pradesh, India
e-mail: rkchitranshi@rediffmail.com

D. Singh
Animal Facility Division, CSIR-IITR, Lucknow,
Uttar Pradesh, India
e-mail: drdharendra@gmail.com

R. Sethumadhavan
School of Bio Sciences and Technology, Vellore
Institute of Technology, Vellore 632014 Tamil Nadu, India
e-mail: rsethumadhavan@vit.ac.in

Keywords Pyrethroids · Cypermethrin · Prenatal · CYPs ·
Expression · Imprinting

Introduction

Cypermethrin, a type II synthetic pyrethroid, widely used in agriculture and public health programs have high affinity for central nervous system (CNS) [1]. Voltage-sensitive sodium and chloride channels are the primary targets of pyrethroids in insects and mammals [2]. At relatively higher concentrations, synthetic pyrethroids are also reported to interact with GABA receptor–ionophore complex leading to neurobehavioral effects including seizures seen with severe type II poisoning [1]. The metabolism of pyrethroids determines to a large extent its neurobehavioral toxicity [1]. As the concentration of pyrethroids increases in the brain, so does the symptoms of neurobehavioral toxicity [3]. Ray and Forshaw [4] have also shown that the proportion of sodium channels modified by pyrethroids is dose dependent.

Age-dependent differences have been reported in the susceptibility to pyrethroids, with the neonates and young ones being more sensitive to the neurotoxic effects of pyrethroids [5]. This enhanced sensitivity has been attributed to the age-dependent differences in the kinetics and limited metabolic capability in the neonates and young [6, 7]. Covalent binding of cypermethrin and other pyrethroids with liver microsomes have indicated the role of cytochrome P450s (CYPs) in the metabolism of pyrethroids [6, 7]. A previous study from our laboratory has shown that while pretreatment with CYP inducers potentiated the symptoms of deltamethrin, another type II pyrethroid insecticide, induced neurobehavioral toxicity, pretreatment with cobalt chloride, an inhibitor of CYP catalysed reactions protected against the neurobehavioral toxicity of deltamethrin [8].

Reports of exposure of pyrethroids during pregnancy and early childhood have prompted studies to investigate the developmental neurotoxicity of pyrethroids [9, 10]. Exposure to pyrethroids during early period of development, particularly during the ‘growth spurt’ phase, has been shown to induce alterations in dopaminergic and cholinergic neurotransmission that may lead to functional delay in the brain [11, 12]. Preliminary studies from our laboratory have shown that deltamethrin affects the ontogeny of hepatic and cerebral xenobiotic metabolizing CYPs [13, 14]. Further, the alterations in cerebral CYPs were found to modify the behavioral activity of the exposed offsprings [13, 14]. However, these studies used commercial formulations of pyrethroids that contain CYP modifiers such as solvents and piperonyl butoxide, which may potentiate the neurotoxicity of pyrethroids.

The present study therefore attempted to investigate the effect of low doses of cypermethrin (technical grade) on the alteration of CYPs in the liver and brain of the offsprings during postnatal development. Attempts were also made to correlate the alterations in CYPs with the circulating testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in the exposed offsprings and the accumulation of the cypermethrin in the brain of the prenatally exposed offsprings. As pyrethroids possess the endocrine disrupting properties [15], studies were also initiated to explore the potential of cypermethrin to imprint the expression of CYPs by rechallenging the exposed offspring during adulthood with cypermethrin. Bioinformatic studies were also carried out to determine elements which notably influence the imprinting status.

Experimental Procedures

Animal Treatment

Adult male albino Wistar rats (~12-week old) and female albino Wistar rats (~10-week old) of proven fertility were obtained from the Animal House facility of the Indian

Institute for Toxicology Research (IITR), Lucknow. The animal experimentation was approved by the ethical committee of the IITR, and all the animals were maintained in accordance to the policy laid down by the animal care committee of IITR. Eighty-four (84) female rats were allowed to mate with 28 adult male rats. On day 0 of pregnancy, the pregnant rats (78 numbers) were randomly divided into four groups of 12 animals each. From gestation day (GD) 5 to GD21 animals in group I, II and III received either 1.25- or 2.5- or 5-mg/kg body weight of cypermethrin (technical grade, mixture of isomers, 98 % pure) orally. Animals in group IV served as control and received corn oil in an identical manner. Following birth, the average litter size was adjusted to eight per dam in all the groups with equal number of males and females so that a minimum of 154 male offsprings were available for dose-dependent studies and 54 male offsprings were there to serve as controls. The male offspring born to the control and treated dams were sacrificed on attaining age of 3 or 6 or 9 or 12 weeks. The liver and brain were immediately removed and processed for isolation of microsomes and total RNA. Prior to sacrifice, the offsprings were monitored for spontaneous locomotor activity. The tissues were processed for isolation of RNA using Trizol (Life Technologies, USA) using the manufacturer’s protocol. The microsomes were prepared from the brain and liver samples as described [16]. Protein content of the samples was estimated by the method of [17].

In another experiment, the male offsprings (12 weeks old) born to the control or 5-mg/kg treated dams were further divided into two groups of 12 offsprings each. The offsprings born to control dams were further divided into two groups of six animals each and received cypermethrin (10 mg/kg body weight; p.o.) for six consecutive days or corn oil. Likewise, the offsprings raised from treated dams were also divided into two groups and received cypermethrin (10 mg/kg body weight; p.o.) for six consecutive days or corn oil. The offsprings were sacrificed 24 h after the last dose. The dose of 10 mg/kg used for rechallenge was based on our pilot study using different doses of 5-, 10- and 20-mg/kg body weight in adult rats. Based on the alterations in the mRNA and protein expression of CYPs, the dose of 10 mg/kg was used for rechallenge (data not shown).

Behavioral Study

The offsprings born to control or cypermethrin-treated mothers were monitored for spontaneous locomotor activity at 3 or 6 or 9 or 12 weeks using a computerized Optovarimex Apple II System as described earlier [14]. Animals to be tested were placed in the activity monitor cage and allowed to acclimatize for a period of 1 min. The motor activity in terms of distance traveled, resting time, stereotypic count and number of rearings were then recorded for

5 min. The monitor cages were subsequently swabbed with 50 % alcohol to prevent interference from animal odors.

Enzymatic Assays

The activity of CYP1A1/1A2-dependent 7-ethoxyresorufin-*O*-deethylase (EROD) or CYP2B1/2B2-dependent 7-pentoxoresorufin-*O*-dealkylase (PROD) was determined in the brain or liver microsomes as reported earlier. CYP2E1-dependent *N*-nitrosodimethylamine demethylase (NDMA-d) activity in the brain and liver microsomes was assayed as described earlier [18].

Immunoblot Analysis

CYP1A1/1A2, 2B1/2B2 and 2E1 isoenzymes were detected by Western blot analysis in microsomes prepared from the liver or brain isolated from control offspring or offspring prenatally exposed to different doses of cypermethrin as described earlier [8, 14]. Liver microsomes isolated from the young rats (approximately 4 weeks old) treated i.p. with 3-methylcholanthren, MC (30 mg/kg body weight suspended in corn oil for 5 days or, phenobarbital (PB) (80 mg/kg body weight dissolved in normal saline five consecutive days) or ethanol (a single i.p. dose of 0.8 mL/kg body weight of ethanol) served as positive controls for CYP1A1/1A2, 2B1/2B2 and 2E1 isoenzymes, respectively. Prior to studying the protein expression of CYPs, the expression with beta-actin was used for normalization.

Quantitative RT-PCR Analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) for the different CYP isoenzymes was carried out as described earlier [19]. The sequences of primers used for CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2E1 and GAPDH have been described by [20]. For absolute quantification, the number of copies of mRNA was calculated by interpolation from the standard curve generated using known amount of full-length cDNA clone of CYP1A1, CYP1A2, CYP2B1, CYP2B2 and CYP2E1, respectively.

Measurement of Serum Testosterone, LH and FSH Levels

Serum hormone levels were assayed using the kits procured from DRG International, Inc., USA (DRG® Testosterone ELISA code EIA-1559, DRG® LH code EIA-1289, DRG® FSH code EIA-1785) according to the manufacturer's protocol.

Gas Chromatography Analysis

The gas chromatography analysis was carried out by the method of Wielgomas and J. Krechniak [21]. The method

was revalidated using three replications of the samples of all the three doses (1.25, 2.5 and 5 mg/kg body weight of cypermethrin) at 3, 6, 9 and 12 weeks to assess the precision of the method.

Computational Sequence Analysis

Computational analysis was carried to identify candidate imprinted genes to allow their imprinting status to be determined experimentally. For the initial analysis, known imprinted genes such as insulin-like growth factor 2 receptor (Igf2r) and imprinted maternally expressed transcript (non-protein coding) (H19), non-imprinted genes like acrosin binding protein (Acrbp) and sperm associated antigen 1 (Spag1) [22] and a group of xenobiotic metabolizing CYPs (CYP1A1/1A2, 2B1/2B2 and 2E1) were selected. The gene sequences for the respective genes were obtained from Ensembl (<http://www.ensembl.org>). For the CpG dinucleotide analysis and estimation of the GC content, Bit Gene Analysis (http://www.bitgene.com/cgi/gene_analysis.cgi) was used. Similar procedures were adopted using Repeat Masker (<http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker>) for the analysis of short interspersed transposable element (SINE) Alus and mammalian-wide interspersed repeat (MIR). The distributions of Alus and MIRs in imprinted genes were analyzed as MIRs are ancient and Alus are more recent evolutionary acquisitions that are excluded from imprinted regions. This exclusion is because of the fact that SINEs can be unusually rich in CpG islands and thus comprise a major target for genomic methylation.

Statistical Analysis

All values are presented as mean \pm S.E.M. Main effects of treatment on enzymatic assays, qRT-PCR, testosterone, LH and FSH levels, gas chromatography analysis and neuro-behavioral parameters were ascertained using the three-way analysis of variance (ANOVA) considering treatment, dose and time as dependent variables and each parameter separately as dependent variable. Prior to the ANOVA, normality of assumptions of data and homogeneity of variance between the groups was ascertained. A post hoc analysis for comparing the two groups was done by calculating the least significant differences at 5 % level of significance using residual mean squares for calculating the *t* values.

Results

Enzyme Activity

Oral administration of different doses of cypermethrin (1.25 or 2.5 or 5 mg/kg) to the pregnant rats produced a significant

dose dependent increase in the activity of EROD, PROD and NDMA-d in the liver microsomes isolated from offsprings, prenatally exposed to 2.5 or 5.0 mg/kg body weight of cypermethrin, postnatally at 3 and 6 weeks. A maximum increase in the enzyme activity was observed at 3 weeks and then the magnitude of increase declined during postnatal development. Though the increase in the activity of EROD, PROD and NDMA-d persisted up to adulthood in the offsprings exposed prenatally to 5 mg/kg of cypermethrin, the increase in the activity was found to be significant only up to 9 weeks (Table 1). Likewise, the activity of EROD, PROD and NDMA-d was found to increase in the brain microsomes isolated from the exposed offspring, though the induction observed were of much lesser magnitude when compared to the liver. The increase in the activity of cerebral EROD, PROD and NDMA-d was found to be significant at 3 weeks in the offsprings, exposed prenatally to 2.5 or 5 mg/kg of cypermethrin and persisted postnatally up to 6 weeks in the offsprings exposed prenatally to 5 mg/kg of cypermethrin (Table 1).

Immunoblot Analysis

The Western blot analysis of the liver microsomes isolated from offsprings born to mothers exposed orally to different doses of cypermethrin (1.25 or 2.5 or 5 mg/kg) produced a dose-dependent increase in the cross-reactivity with the polyclonal antibody raised against rat liver CYP1A1/1A2, 2B1/2B2 and 2E1 isoenzymes during postnatal development (Fig. 1). Densitometric analysis revealed a maximum increase in the cross-reactivity in the offsprings postnatally at 3 weeks of age while no significant change was observed in the liver microsomes isolated from exposed offsprings postnatally at 12 weeks at any of the doses (Fig. 2). Western blot analysis with polyclonal antibody raised against beta-actin did not show any changes in the expression of microsomes isolated from control or treated offsprings (Fig. 3a). A significant increase in the immunoreactivity comigrating with rat liver CYP1A1/1A2 or 2B1/2B2 or 2E1 in the liver microsomes prepared from offsprings exposed prenatally to 2.5 or 5 mg/kg of cypermethrin was observed postnatally at 3- and 6 weeks which persisted up to 9 weeks in the offsprings prenatally exposed to 5 mg/kg of cypermethrin (Fig. 2).

A relatively smaller but distinct increase in the immunoreactivity was observed in the microsomal proteins prepared from the brain isolated from offsprings raised on mothers receiving 2.5 or 5 mg/kg of cypermethrin, postnatally at 3 weeks (Fig. 1). A densitometric analysis revealed that prenatal exposure to 2.5 or 5 mg/kg led to an increase in the immunoreactivity, comigrating with rat liver CYP1A1/1A2 or CYP2B1/2B2 or CYP2E1 in the microsomes prepared from the brain isolated from the offsprings postnatally at 3 weeks.

The increase in the immunoreactivity persisted up to 9 weeks, though the increase was significant only up to 6 weeks in the offsprings exposed to 5 mg/kg of cypermethrin (Fig. 2).

qRT-PCR analysis

Absolute quantification studies using qRT-PCR revealed a significant increase in the mRNA expression of CYP1A1, CYP2B1 and CYP2B2 in the liver isolated from offsprings exposed prenatally to 2.5 or 5 mg/kg of cypermethrin during postnatal development at 3 and 6 weeks. The increase was found to persist in the liver up to 9 weeks in the offsprings exposed prenatally to 5 mg/kg of cypermethrin (Table 2). Likewise, a statistically significant dose-dependent increase in the mRNA expression of CYP1A2 and CYP2E1 isoenzymes was observed in the liver isolated from offsprings exposed prenatally to 2.5 or 5 mg/kg during postnatal development at 3, 6 and 9 weeks (Table 2).

A statistically significant dose-dependent increase was also observed in the mRNA expression of CYP1A1, CYP1A2 and CYP2B1 in the brain isolated from offsprings exposed prenatally to 2.5 and 5 mg/kg of cypermethrin postnatally up to 3 and 6 weeks and up to 9 weeks in the offsprings exposed prenatally to 5 mg/kg. The cerebral mRNA expression of CYP2B2 and CYP2E1 was also found to be significantly increased postnatally at 3 and 6 weeks in the offsprings exposed prenatally to 2.5 or 5 mg/kg of cypermethrin (Table 2).

Accumulation of Pyrethroid in the Brain of Prenatally Exposed Rat Offsprings

A GC-ECD analysis of brain samples isolated from offsprings prenatally exposed to different doses of cypermethrin revealed a dose-dependent accumulation of unmetabolized cypermethrin in the brain during postnatal development, with maximum accumulation occurring in the brain of exposed offsprings postnatally at 3 weeks of age (Table 3). The levels of cypermethrin accumulating at the dose of 5 mg/kg of cypermethrin in the brain were about 0.70 % of the dose administered (Table 3). The accumulation of cypermethrin then subsequently declined during postnatal development, and very low levels of unmetabolized cypermethrin could be detected postnatally at 6 and 9 weeks of age (Table 3).

Circulating Hormone Levels in the Prenatally Exposed Offsprings

Prenatal exposure of cypermethrin produced a dose-dependent decrease in the circulating testosterone levels in the prenatally exposed offsprings with the effects being statistically significant postnatally at 3 and 6 weeks in

Table 1 Effect of prenatal exposure of different doses of cypermethrin on the activity of the brain and liver CYP enzymes in offsprings during postnatal development

Dose (mg/kg)	EROD ^a				PROD ¹				NDMA-d ^b			
	Liver		Brain		Liver		Brain		Liver		Brain	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
3 weeks												
1.25	11.1 ± 1.1	12.8 ± 1.15	0.44 ± 0.04	0.45 ± 0.45	5.60 ± 0.53	6.49 ± 0.6	0.47 ± 0.05	0.49 ± 0.05	0.91 ± 0.08	1.07 ± 0.10	0.42 ± 0.04	0.43 ± 0.04
2.5	11.3 ± 0.6	17.9 ± 1.1*	0.48 ± 0.29	0.58 ± 0.02*	5.77 ± 0.38	7.93 ± 0.4*	0.46 ± 0.04	0.57 ± 0.05*	1.05 ± 0.05	2.03 ± 0.122*	0.47 ± 0.02	0.58 ± 0.02*
5.0	11.5 ± 0.7	20.8 ± 1.3*	0.43 ± 0.02	0.57 ± 0.03*	5.85 ± 0.58	10.2 ± 0.5*	0.43 ± 0.04	0.57 ± 0.03*	1.20 ± 0.06	2.70 ± 0.17*	0.43 ± 0.02	0.59 ± 0.03*
6 weeks												
1.25	46.4 ± 4.4	50.5 ± 5.0	0.49 ± 0.04	0.50 ± 0.05	13.7 ± 1.29	14.4 ± 1.4	0.52 ± 0.05	0.53 ± 0.05	1.35 ± 0.13	1.54 ± 0.15	0.53 ± 0.05	0.53 ± 0.05
2.5	46.0 ± 2.7	57.5 ± 2.8*	0.51 ± 0.05	0.58 ± 0.05	13.2 ± 0.78	16.1 ± 0.8*	0.51 ± 0.04	0.56 ± 0.05	1.36 ± 0.07	2.45 ± 0.15*	0.55 ± 0.05	0.57 ± 0.05
5.0	45.7 ± 3.7	68.5 ± 4.1*	0.52 ± 0.03	0.63 ± 0.03*	12.9 ± 0.71	19.0 ± 1.0*	0.50 ± 0.03	0.61 ± 0.03*	1.33 ± 0.07	2.28 ± 0.12*	0.58 ± 0.03	0.71 ± 0.34*
9 weeks												
1.25	48.2 ± 4.6	49.4 ± 4.9	0.53 ± 0.05	0.53 ± 0.05	15.2 ± 1.45	15.6 ± 1.5	0.56 ± 0.52	0.56 ± 0.04	1.55 ± 0.15	1.64 ± 0.15	0.59 ± 0.05	0.59 ± 0.06
2.5	47.8 ± 4.5	53.5 ± 5.2	0.52 ± 0.05	0.53 ± 0.05	15.1 ± 1.42	16.7 ± 1.6	0.55 ± 0.05	0.56 ± 0.05	1.52 ± 0.13	1.79 ± 0.17	0.58 ± 0.06	0.59 ± 0.05
5.0	47.3 ± 2.6	56.8 ± 2.8*	0.51 ± 0.05	0.57 ± 0.05	14.9 ± 1.42	18.2 ± 0.9*	0.52 ± 0.05	0.53 ± 0.05	1.50 ± 0.08	1.92 ± 0.09*	0.58 ± 0.05	0.63 ± 0.06
12 weeks												
1.25	53.6 ± 5.0	53.8 ± 5.1	0.54 ± 0.04	0.54 ± 0.05	16.8 ± 1.58	16.9 ± 1.6	0.61 ± 0.05	0.61 ± 0.06	1.58 ± 0.15	1.59 ± 0.15	0.60 ± 0.06	0.60 ± 0.06
2.5	51.7 ± 5.0	53.7 ± 5.2	0.54 ± 0.05	0.55 ± 0.05	16.5 ± 1.57	17.2 ± 1.7	0.61 ± 0.06	0.62 ± 0.06	1.61 ± 0.15	1.64 ± 0.16	0.60 ± 0.06	0.61 ± 0.05
5.0	50.5 ± 5.0	54.1 ± 5.4	0.54 ± 0.04	0.56 ± 0.05	16.4 ± 1.61	17.4 ± 1.7	0.62 ± 0.06	0.64 ± 0.06	1.60 ± 0.16	1.64 ± 0.17	0.59 ± 0.05	0.60 ± 0.05

All the values represent mean ± S.E.M. of six samples

* $p < 0.05$ when compared to control

^a Specific activity in picomoles resorufin/minute/milligram protein

^b Specific activity in nanomoles formaldehyde/ minute/milligram protein

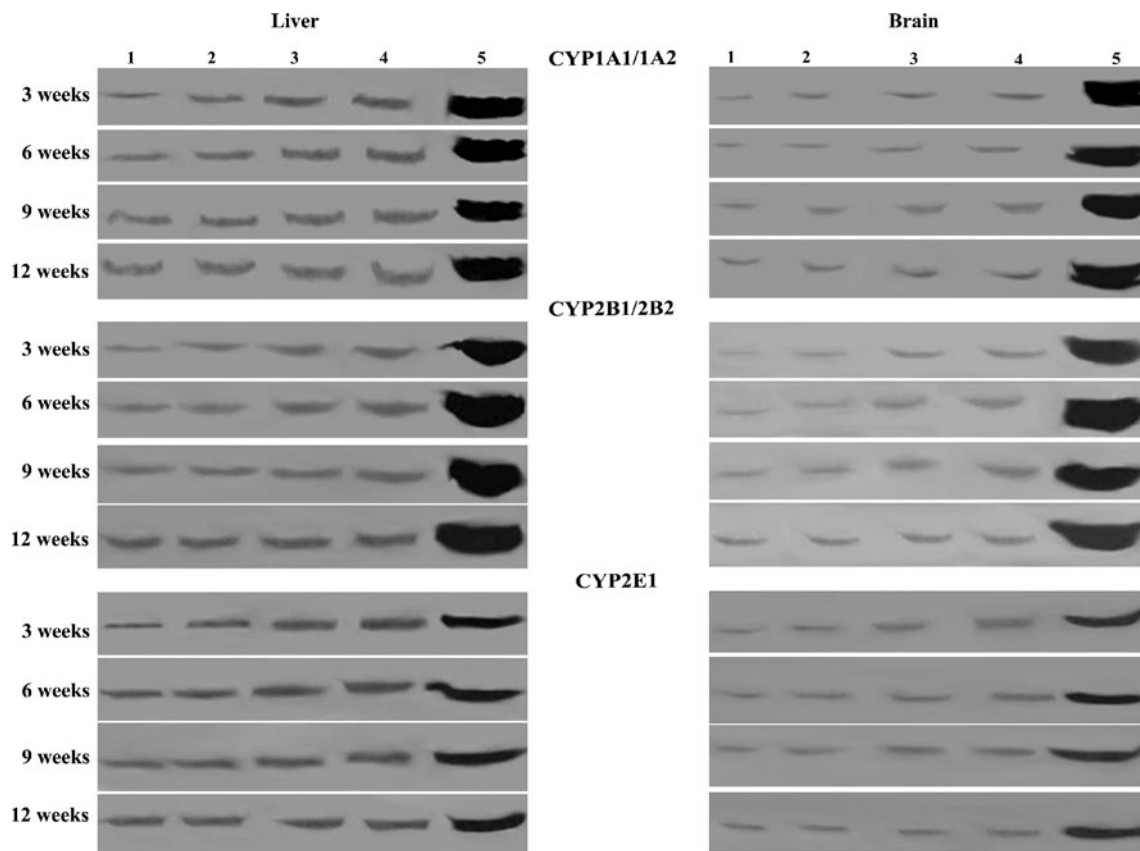


Fig. 1 Western blot analysis of microsomal proteins isolated from prenatally exposed offsprings with anti-CYP1A1/1A2 or 2B1/2B2 or CYP2E1. Lane 1- microsomal proteins from liver or brain of offsprings raised on control rat mothers. Lanes 2-4- microsomal proteins prepared from liver (25 μ g) or brain (250 μ g) of rat offsprings exposed

prenatally to 1.25, 2.5 and 5 mg/kg of cypermethrin respectively. Lane 5- 25 μ g of microsomal proteins isolated from liver of rats pretreated with different CYP inducers—MC for CYP1A1/1A2 or PB for CYP 2B1/2B2 or ethanol for CYP2E1

offsprings exposed prenatally to 2.5 and 5 mg/kg of cypermethrin and up to 9 weeks of age in the offsprings exposed prenatally to 5 mg/kg of cypermethrin (Table 4).

A dose-dependent increase in the levels of serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels was observed in the exposed offsprings when compared to the levels observed in the offspring nursing on control mothers. The increase observed in the levels of serum LH and FSH were found to be statistically significant in the offsprings exposed prenatally to 2.5 and 5 mg/kg of cypermethrin postnatally at 3 and 6 weeks of age, and up to 9 weeks of age in the offsprings exposed prenatally to 5 mg/kg of cypermethrin (Table 4).

Spontaneous Locomotor Activity in Prenatally Exposed Offsprings

A three-way ANOVA, considering treatment dose and time as dependent variables, revealed a significant decrease in the distance traveled, stereotypic count and number of rearing, whereas the resting time, inversely related to the distance traveled, was found to be increased significantly postnatally

at 3 and 6 weeks in the offsprings prenatally exposed to 2.5 and 5 mg/kg of cypermethrin. The decrease in the stereotypic count and number of rearing movements was also found to be statistically significant postnatally at 9 weeks in the offsprings prenatally exposed to 5 mg/kg of cypermethrin (Table 5). The decrease observed in the distance traveled was found to be statistically significant even up to 9 weeks in the offspring prenatally exposed to 5 mg/kg of cypermethrin (Table 5).

Computational Sequence Analysis

A computational sequence analysis of region upstream to the coding sequences, the coding region and the region upstream to the coding sequences in *Igf2r* and *H19*, the genes which are known to be imprinted, revealed no SINE–Alu and SINE–MIR repeats (Table 6). In contrast, sequence analysis of *Acbrp* and *Spag1* genes, which are not imprinted, demonstrated SINE–Alu and SINE–MIR elements in the above-mentioned regions. A computational analysis of xenobiotic metabolizing CYPs (CYP1A-, 2B- and 2E1) shows the complete absence of SINE–Alu and SINE–MIR elements in the regions mentioned

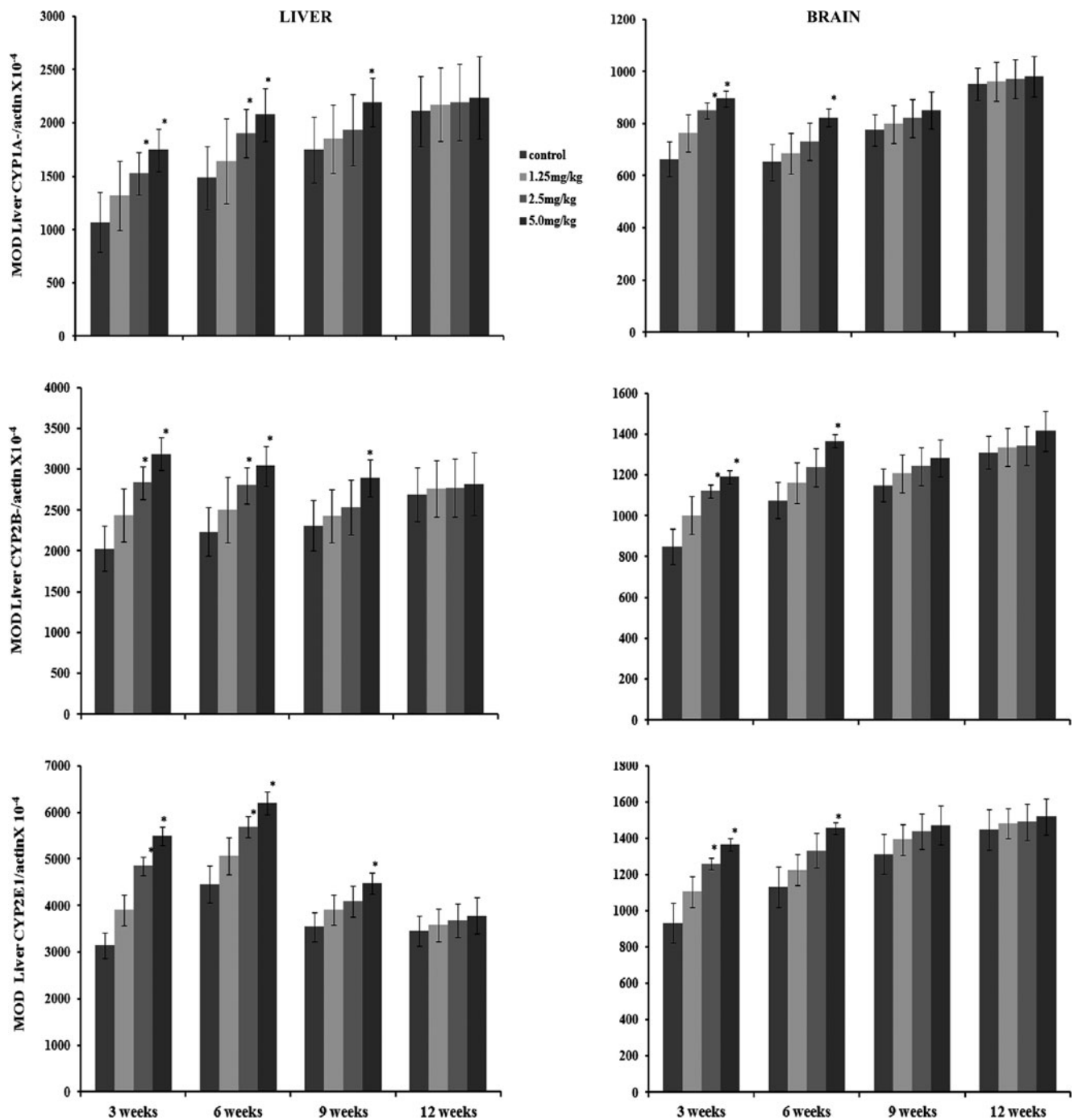


Fig. 2 Densitometric analysis of Western blots of rat liver and brain microsomal proteins with anti-CYP1A1/1A2 or 2B1/2B2 or CYP2E1. Bars represent dose-dependent effects of prenatal exposure to different

doses of cypermethrin at 3, 6, 9 or 12 weeks postnatally. All the values represent mean \pm S.E.M. of three experiments; * $P < 0.05$; MOD-Mean Optical Density

above (Table 6). The sequence analysis of *Igf2r*, *H19* (imprinted), *Acbrp*, *Spag1* (non-imprinted) and *CYP* genes, revealed no conclusive alterations in the content for CpG islands and GC content in the regions mentioned above (Table 6). Similar results were observed with other imprinted genes (*Igf2*, *Rasgrf1* and *Sfmbt2*) and non-imprinted genes (*Ggnbp2*, *Tpi1* and *Cd46*) data not shown.

Postnatal Effects of Cypermethrin on Cerebral and Hepatic CYPs in the Offspring Prenatally Exposed to Cypermethrin

A Western blot analysis of liver microsomes of rat offsprings born to control dams or dams exposed prenatally to cypermethrin (5.0 mg/kg) and subsequently treated postnatally at 12 weeks to cypermethrin (10 mg/kg body weight; orally)

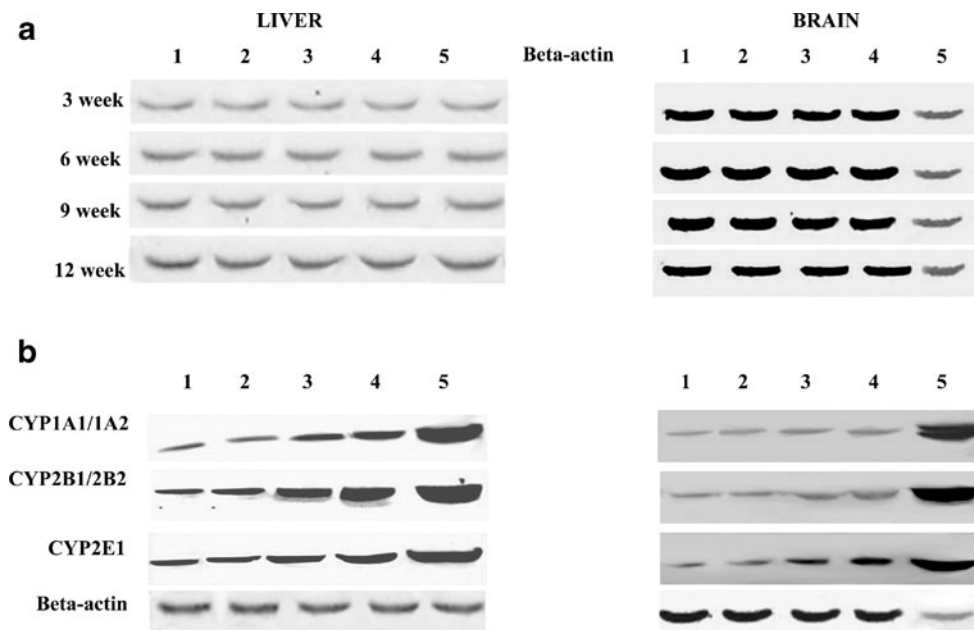


Fig. 3 Western blot analysis of liver and brain microsomal proteins isolated from (**3a**) control and prenatally exposed offsprings with anti- β -actin or (**3b**) control and prenatally exposed offsprings rechallenged with cypermethrin during adulthood. **3a**: Lane 1- microsomal proteins from liver or brain of offsprings raised on control rat mothers. Lanes 2–4- microsomal proteins prepared from liver (25 μ g) or brain (250 μ g) of rat offsprings exposed prenatally to 1.25, 2.5 and 5 mg/kg of cypermethrin respectively. Lane 5- 25 μ g of microsomal proteins isolated from liver of rats pretreated with different CYP inducers—

MC for CYP1A1/1A2 or PB for CYP 2B1/2B2 or ethanol for CYP2E1. **3B**: Lanes 1–2- microsomal proteins prepared from liver (25 μ g) or brain (250 μ g) of 12 weeks old offsprings raised on control dams or dams treated with cypermethrin during gestation respectively. Lanes 3–4- microsomal proteins prepared from liver (25 μ g) or brain (250 μ g) of 12 weeks old offsprings raised on control dams or dams treated with cypermethrin during gestation and subsequently rechallenged with cypermethrin respectively. Lane 5- microsomal proteins isolated from rats treated with different CYP inducers

for 6 consecutive days demonstrated an increase in the cross-reactivity with the polyclonal antibody raised against rat liver CYP1A1/1A2 or CYP2B1/2B2 or CYP2E1, when compared to the controls (Fig. 3b). Densitometric analysis revealed that this increase in the protein expression of CYPs was found to be of much higher magnitude in microsomes isolated from offsprings exposed prenatally to cypermethrin and subsequently rechallenged postnatally at 12 weeks with cypermethrin, when compared with the offsprings raised on control dams and treated postnatally at 12 weeks with cypermethrin (data not shown). A similar pattern of increase in the protein expression of CYP1A1/1A2 or CYP2B1/2B2 or CYP2E1 isoenzymes was observed in the brain microsomes isolated from rat offspring born to control dams and treated with cypermethrin postnatally at adulthood with cypermethrin or offsprings exposed prenatally to cypermethrin and rechallenged with cypermethrin postnatally at 12 weeks of age (Fig. 3b).

Absolute quantification studies using qRT-PCR revealed a significant increase in the mRNA expression of CYP1A1, CYP1A2, CYP2B1, CYP2B2 and CYP2E1 in the liver isolated from offsprings born to control dams and treated with cypermethrin postnatally at 12 weeks when compared to the offsprings nursing on control dams. A significant increase in the mRNA expression of these CYP isoenzymes

was also observed in the liver isolated from offsprings exposed prenatally to cypermethrin and subsequently rechallenged with cypermethrin postnatally during adulthood (12 weeks old) when compared to the offsprings nursing on control dams. The increase observed in the expression of the CYP isoenzymes was of much higher magnitude when compared to the offsprings nursing on control dams and treated with cypermethrin postnatally at 12 weeks of age (Table 7).

Discussion

The present data indicating dose-dependent effects of cypermethrin on the postnatal development of CYPs in liver and brain have provided support to our previous studies [13, 14], carried out with the commercial pyrethroid (deltamethrin) formulation, that low doses of pyrethroids have the potential to influence the ontogeny of cerebral CYPs, which in turn could influence the closely associated physiological functions in the brain [23]. As reported earlier [14], the alterations in the postnatal development of CYPs in the brain and liver were found to persist up to adulthood and were found to be associated with significant changes in the spontaneous locomotor activity (SLA) in the exposed rat offsprings.

Table 2 Effect of prenatal exposure of different doses of cypermethrin on mRNA expression of CYPs in the liver and brain of rat offsprings during postnatal development

	CYPIA1							
	Liver (X10 ⁵)				Brain (X10 ³)			
	Control	1.25 mg/kg	2.5 mg/kg	5.0 mg/kg	Control	1.25 mg/kg	2.5 mg/kg	5.0 mg/kg
3 weeks	2.46 ± 0.2	2.52 ± 0.2	3.98 ± 0.2*	4.46 ± 0.3*	9.02 ± 0.7	9.11 ± 0.8	12.6 ± 0.7*	13.5 ± 0.7*
6 weeks	2.76 ± 0.2	2.80 ± 0.3	3.86 ± 0.2*	4.21 ± 0.2*	9.39 ± 0.7	9.40 ± 0.9	11.7 ± 0.6*	12.7 ± 0.6*
9 weeks	2.88 ± 0.3	2.91 ± 0.3	3.44 ± 0.3	3.86 ± 0.2*	9.59 ± 0.7	9.60 ± 0.9	11.4 ± 1.1	12.2 ± 0.7*
12 weeks	3.00 ± 0.3	3.03 ± 0.3	3.21 ± 0.3	3.62 ± 0.3	9.40 ± 0.8	9.40 ± 0.9	9.64 ± 0.9	10.1 ± 0.9
	CYP1A2							
3 weeks	9.60 ± 0.9	10.6 ± 1.0	23.8 ± 1.4*	25.5 ± 1.5*	0.52 ± 0.04	0.54 ± 0.05	0.89 ± 0.05*	1.06 ± 0.05*
6 weeks	11.65 ± 1.1	12.2 ± 1.2	24.1 ± 1.5*	27.6 ± 1.6*	0.76 ± 0.06	0.77 ± 0.07	1.08 ± 0.06*	1.19 ± 0.05*
9 weeks	12.50 ± 1.2	12.7 ± 1.3	19.9 ± 1.2*	24.1 ± 1.3*	1.32 ± 0.11	1.33 ± 0.13	1.57 ± 0.15	1.75 ± 0.09*
12 weeks	12.80 ± 1.3	12.9 ± 1.3	13.8 ± 1.3	15.2 ± 1.5	1.35 ± 0.13	1.35 ± 0.13	1.42 ± 0.14	1.52 ± 0.15
	CYP2B1							
3 weeks	0.84 ± 0.1	0.90 ± 0.1	1.72 ± 0.1*	1.91 ± 0.1*	1.66 ± 0.1	1.77 ± 0.2	2.54 ± 0.2*	3.10 ± 0.2*
6 weeks	1.56 ± 0.2	1.64 ± 0.2	2.53 ± 0.2*	2.61 ± 0.2*	2.12 ± 0.2	2.16 ± 0.2	2.98 ± 0.2*	3.24 ± 0.2*
9 weeks	1.44 ± 0.2	1.44 ± 0.1	1.69 ± 0.2	1.83 ± 0.1*	2.10 ± 0.2	2.12 ± 0.2	2.39 ± 0.2	2.59 ± 0.3*
12 weeks	1.25 ± 0.1	1.25 ± 0.1	1.43 ± 0.1	1.48 ± 0.1	2.00 ± 0.2	2.00 ± 0.2	2.02 ± 0.2	2.26 ± 0.2
	CYP2B2							
3 weeks	3.84 ± 0.3	4.15 ± 0.4	8.50 ± 0.5*	9.45 ± 0.6*	2.79 ± 0.2	2.81 ± 0.3	3.79 ± 0.2*	4.10 ± 0.2*
6 weeks	3.91 ± 0.4	4.03 ± 0.4	6.98 ± 0.4*	7.89 ± 0.5*	2.94 ± 0.2	2.95 ± 0.3	3.58 ± 0.2*	3.86 ± 0.2*
9 weeks	4.64 ± 0.4	4.70 ± 0.5	5.80 ± 0.5	6.35 ± 0.3*	2.91 ± 0.3	2.92 ± 0.3	3.18 ± 0.3	3.48 ± 0.3
12 weeks	4.72 ± 0.5	4.75 ± 0.5	4.82 ± 0.5	5.67 ± 0.6	3.00 ± 0.3	3.00 ± 0.3	3.04 ± 0.3	3.10 ± 0.3
	CYP2E1							
3 weeks	2.86 ± 0.2	3.17 ± 0.3	6.30 ± 0.4*	7.00 ± 0.4*	2.21 ± 0.2	2.38 ± 0.2	3.32 ± 0.2*	4.12 ± 0.2*
6 weeks	3.82 ± 0.3	3.97 ± 0.4	6.25 ± 0.3*	7.25 ± 0.4*	2.31 ± 0.2	2.36 ± 0.2	3.25 ± 0.2*	3.43 ± 0.2*
9 weeks	3.94 ± 0.3	3.97 ± 0.4	5.24 ± 0.3*	5.85 ± 0.3*	2.55 ± 0.2	2.56 ± 0.2	2.73 ± 0.3	3.00 ± 0.3
12 weeks	4.00 ± 0.4	4.00 ± 0.4	4.16 ± 0.3	4.92 ± 0.5	2.76 ± 0.2	2.76 ± 0.2	2.81 ± 0.3	2.90 ± 0.3

All the values are mean ± S.E. of six animals. Ct values of each sample was normalized with Ct value of endogenous control. Copy numbers of CYP1A1, 1A2, 2B1, 2B2 and 2E1 mRNA per microgram of total mRNA

* $p < 0.05$ when compared to the controls

Further, dose-dependent accumulation of cypermethrin in the brain of prenatally exposed offsprings is consistent with the previous reports indicating placental transfer of pyrethroids that eventually leads to the accumulation of cypermethrin in the brain of exposed offsprings [24, 25]. Even

though the metabolites of cypermethrin were not identified in the present study, it has been found that metabolites of cypermethrin accumulated in the brain of exposed offsprings even up to 12 weeks (unpublished observation from our group). There are also reports suggesting that

Table 3 Dose dependent accumulation of cypermethrin in offsprings exposed prenatally to different doses of cypermethrin

	Concentration (µg/g)			
	3 weeks	6 weeks	9 weeks	12 weeks
1.25 mg/kg	0.007 ± 0.0007	0.004 ± 0.0004	0.002 ± 0.0002	0.001 ± 0.0009
2.5 mg/kg	0.015 ± 0.0009*	0.010 ± 0.0010	0.005 ± 0.0004	0.003 ± 0.0003
5.0 mg/kg	0.035 ± 0.0018*	0.030 ± 0.0017*	0.012 ± 0.0011	0.006 ± 0.0006

All the values are mean ± SE of six animals

* $p < 0.05$ when compared with the controls

Table 4 Effect of prenatal exposure of different doses of cypermethrin on hormone levels in offsprings during postnatal development

	Testosterone			
	3 weeks	6 weeks	9 weeks	12 weeks
Control	1.78 ± 0.12	2.67 ± 0.24	2.89 ± 0.27	3.11 ± 0.30
1.25 mg/kg	1.38 ± 0.13	2.32 ± 0.22	2.76 ± 0.27	3.04 ± 0.29
2.5 mg/kg	0.92 ± 0.05*	1.75 ± 0.10*	2.47 ± 0.24	2.90 ± 0.28
5.0 mg/kg	0.71 ± 0.03*	1.41 ± 0.08*	2.08 ± 0.10*	2.71 ± 0.26
	LH			
Control	1.88 ± 0.15	2.83 ± 0.25	3.08 ± 0.29	3.29 ± 0.31
1.25 mg/kg	2.33 ± 0.22	3.24 ± 0.29	3.26 ± 0.32	3.39 ± 0.32
2.5 mg/kg	2.91 ± 0.12*	3.98 ± 0.19*	3.63 ± 0.35	3.54 ± 0.34
5.0 mg/kg	3.21 ± 0.15*	4.42 ± 0.24*	4.03 ± 0.24*	3.90 ± 0.38
	FSH			
Control	2.10 ± 0.19	3.02 ± 0.26	3.41 ± 0.33	3.94 ± 0.37
1.25 mg/kg	2.63 ± 0.25	3.53 ± 0.33	3.65 ± 0.36	4.08 ± 0.37
2.5 mg/kg	3.49 ± 0.18*	4.38 ± 0.22*	4.13 ± 0.39	4.29 ± 0.41
5.0 mg/kg	3.93 ± 0.19*	5.07 ± 0.25*	4.84 ± 0.28*	4.73 ± 0.46

All values are in nanograms per milliliter and are mean ± SE of six animals

* $p < 0.05$ when compared with the controls

pyrethroids might be accumulating in the fatty tissues in the mothers, which in turn may partition in the mothers' milk leading to further exposure of the cypermethrin to offsprings during lactation [1]. Thus, continued exposure of the prenatally exposed offsprings to pyrethroid, associated with low levels of expression of the CYPs during early postnatal development, may have led to the accumulation of cypermethrin in the brain to levels sufficient to induce

alterations in SLA and possibly account for persistence in increase in the CYPs in the brain and liver during postnatal development.

As only a minute fraction of the dose of cypermethrin administered to the mothers was identified in the brain of the offsprings and due to the endocrine disrupting ability of cypermethrin [15], the persistence in the increase in expression of CYPs could possibly be attributed to the imprinting of

Table 5 Dose-dependent effect of gestational exposure of cypermethrin on spontaneous locomotor activity of offsprings postnatally

Dose (mg/kg)	Distance traveled (cm)		Resting time (s)		Stereotypic count		Number of rearing	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
3 weeks								
1.25	1,293.8 ± 103.5	970.40 ± 90.2	107.5 ± 8.6	134.4 ± 13.2	23.6 ± 2.1	18.9 ± 1.8	12.0 ± 1.1	9.80 ± 0.90
2.5	1,073.3 ± 54.60	526.30 ± 26.3*	114.3 ± 5.2	172.6 ± 4.00*	22.0 ± 1.1	11.1 ± 0.5*	10.9 ± 0.5	5.70 ± 0.30*
5.0	1,137.0 ± 68.20	436.30 ± 28.4*	109.4 ± 5.3	177.2 ± 5.20*	25.8 ± 1.2	10.3 ± 0.5*	10.6 ± 0.5	4.30 ± 0.20*
6 weeks								
1.25	1,275.0 ± 108.4	1,083.80 ± 102.5	157.5 ± 12.9	181.1 ± 16.8	23.3 ± 2.1	21.1 ± 2.1	15.4 ± 1.4	13.6 ± 1.3
2.5	1,515.0 ± 83.30	1,060.5 ± 53.0*	149.4 ± 8.40	194.2 ± 9.90*	26.8 ± 1.5	19.8 ± 1.0*	17.9 ± 0.9	13.1 ± 0.6*
5.0	1,331.0 ± 66.60	603.80 ± 30.2*	127.3 ± 4.30	188.4 ± 4.00*	24.4 ± 1.2	12.7 ± 0.6*	16.3 ± 0.8	8.8 ± 0.4*
9 weeks								
1.25	1,590.0 ± 130.4	1,534.4 ± 138.1	177.6 ± 13.9	183.8 ± 17.1	24.2 ± 1.8	23.7 ± 1.9	17.3 ± 1.6	16.8 ± 1.5
2.5	1,827.5 ± 164.5	1,644.8 ± 161.2	164.6 ± 14.3	181.1 ± 17.7	28.1 ± 1.9	25.9 ± 2.3	18.4 ± 1.7	16.7 ± 1.6
5.0	1,924.0 ± 115.4	1,477.7 ± 78.3*	154.0 ± 9.20	190.7 ± 10.1*	28.0 ± 1.5	21.8 ± 1.1*	19.5 ± 1.1	16.4 ± 0.7*
12 weeks								
1.25	2,020.0 ± 191.9	1,992.0 ± 199.1	160.2 ± 15.4	161.6 ± 16.0	35.5 ± 3.5	35.3 ± 3.5	22.8 ± 2.2	22.6 ± 2.2
2.5	2,056.0 ± 200.5	1,964.7 ± 194.5	164.5 ± 16.1	170.5 ± 16.9	38.4 ± 3.8	37.3 ± 3.6	24.6 ± 2.4	23.8 ± 2.3
5.0	2,075.0 ± 205.0	1,901.9 ± 188.1	162.6 ± 16.0	176.6 ± 17.5	36.7 ± 3.4	34.6 ± 3.4	26.2 ± 2.6	24.7 ± 2.4

All the values represent mean ± S.E.M. of eight animals

* $p < 0.05$ when compared to control

Table 6 The number of SINEs, Alus, MIRs, CpG islands and GC content in upstream, coding and downstream sequences of imprinted genes, non-imprinted and CYP genes

Genes	Upstream				Coding				Downstream			
	SINE–Alu	SINE–MIR	CpG	GC	SINE–Alu	SINE–MIR	CpG	GC	SINE–Alu	SINE–MIR	CpG	GC
<i>Igf2r</i> (imprinted)	Nil	Nil	40	60.67	Nil	Nil	223	51.01	Nil	Nil	11	50.83
<i>H19</i> (imprinted)	Nil	Nil	18	58.76	Nil	Nil	10	59.09	Nil	Nil	19	57.38
<i>CYP1A1</i>	Nil	Nil	8	53.50	Nil	Nil	39	48.95	Nil	Nil	5	44.07
<i>CYP1A2</i>	Nil	Nil	4	46.00	Nil	Nil	40	52.28	Nil	Nil	7	42.50
<i>CYP2B1</i>	Nil	Nil	7	44.41	Nil	Nil	35	50.96	Nil	Nil	6	41.67
<i>CYP2B2</i>	Nil	Nil	6	45.33	Nil	Nil	30	51.06	Nil	Nil	3	41.33
<i>CYP2E1</i>	Nil	Nil	4	45.33	Nil	Nil	27	46.94	Nil	Nil	1	40.33
<i>Acrbp</i> (non-imprinted)	Nil	Nil	16	52.78	7	2	40	56.76	Nil	Nil	5	51.87
<i>Spag1</i> (non-imprinted)	1	Nil	12	46.22	4	Nil	90	49.18	1	Nil	5	44.05

Imprinted and non-imprinted genes were in accordance with Kobayashi et al. (2012)

the hepatic and cerebral CYPs in the exposed offspring. A computational sequence analysis of CYPs along with several genes, including *Igf2r* and *H19*, the imprinted genes and non-imprinted genes, was carried out to determine the elements which influence the imprinting status. Among the several sequence element variables, the number of CpG islands, GC content, presence or absence of SINE–Alu and SINE–MIR are the major sequence characteristics of imprinted genes [26, 27]. As observed with *Igf2r* and *H19*, the bioinformatic analysis demonstrated complete absence of SINE–Alu and SINE–MIR elements in xenobiotic metabolizing CYP1A-, 2B- and 2E1, suggesting that these xenobiotic metabolizing CYPs have the potential to be imprinted following xenobiotic exposure. This

similarity or dissimilarity in the sequence elements with known imprinted and non-imprinted genes was more prominent in the coding region of these CYPs, which is important in assessing the imprinting potential of any gene [28]. Further evidence that prenatal exposure to even low doses of cypermethrin has the potential to imprint the inducibility of CYPs in the offsprings was shown by enhanced responsiveness of the CYP1A1, 2B1 and 2E1 isoenzymes in the brain and liver when rechallenged during adulthood demonstrating that the induction mechanisms of CYPs are imprinted in the exposed offsprings.

The persistence in the increase in expression of xenobiotic metabolizing CYPs could have toxicological consequences as

Table 7 Effect of postnatal exposure of cypermethrin on the mRNA expression of CYPs in the liver and brain of rat offsprings raised on control dams or dams prenatally exposed to cypermethrin

Dose (mg/kg)	Brain			
	Control (Gr. I)	5 mg/kg (Gr. II prenatal)	10 mg/kg (Gr. III postnatal)	5 mg/kg (prenatal) + 10 mg/kg (Gr. IV postnatal)
CYP1A1 ($\times 10^3$)	9.42 ± 1.0	10.05 ± 1.0	13.9 ± 1.3*	21.81 ± 2.1*
CYP1A2 ($\times 10^4$)	Liver			
CYP1A1 ($\times 10^5$)	3.20 ± 0.3	3.65 ± 0.4	5.52 ± 0.5*	8.16 ± 0.8*
CYP1A2 ($\times 10^6$)	12.80 ± 1.3	15.3 ± 1.5	25.22 ± 2.5*	32.3 ± 3.2*
CYP2B1 ($\times 10^5$)	1.25 ± 0.1	1.48 ± 0.1	2.25 ± 0.2*	4.37 ± 0.4*
CYP2B2 ($\times 10^5$)	4.75 ± 0.5	5.70 ± 0.6	8.92 ± 0.9*	14.9 ± 1.5*
CYP2E1 ($\times 10^8$)	4.15 ± 0.4	4.95 ± 0.5	9.60 ± 1.0*	12.0 ± 1.2*
CYP2B1 ($\times 10^4$)	2.10 ± 0.2	2.26 ± 0.2	2.70 ± 0.4*	5.30 ± 0.5*
CYP2B2 ($\times 10^4$)	3.05 ± 0.3	3.36 ± 0.5	6.33 ± 0.6	7.20 ± 0.7*
CYP2E1 ($\times 10^4$)	2.80 ± 0.3	2.90 ± 0.3	3.61 ± 0.4*	4.83 ± 0.4*

All the values are mean ± S.E. of six animals. Ct values of each sample were normalized with Ct value of endogenous control. Copy numbers of CYP1A1, 1A2, 2B1, 2B2 and 2E1 mRNA per microgram of total mRNA

Gr: group

* $p < 0.05$ when compared to the controls

these CYPs are reported to be associated with several physiological functions in the brain. Functional association of CYP2E1 with dopaminergic transporters and dopaminergic neurotransmission [29, 30] suggests that the imprinting of CYP2E1 in exposed offsprings may influence the susceptibility of dopaminergic neurotransmission that may help in explaining the persistence in alterations in SLA even after exposure of mothers to such low doses of cypermethrin (used in the present study), which do not produce any symptoms of overt toxicity or neurobehavioral toxicity in the adults. Recently, it has been shown that early postnatal exposure to cypermethrin enhances the susceptibility of rats to dopaminergic neurodegeneration when rechallenged during adulthood [31]. Likewise, the imprinting of CYP2B1/2B2 or CYP1A1/1A2 isoenzymes in the offsprings following prenatal exposure of cypermethrin may predispose the exposed offsprings to the inhibitory effect of pyrethroids at the picrotoxin binding site of GABA receptor–ionophore complex [32]. Roberge et al. [33] have reported that the binding of the ligands of GABA_A receptor is linked with the transcriptional activation of CYP2B and CYP3A genes. It has been hypothesized that some of the nuclear receptors, that regulate the induction of CYPs, have a flexible ligand binding domain which mimic the binding characteristics of multimeric membrane-bound receptor complexes (such as GABA_A receptor) with binding sites for structurally different chemicals. Similarly, the role of CYP1A isoenzymes in α -adrenoreceptor-dependent signaling pathways is also well established [34].

In summary, the results of the present study have provided evidence that prenatal exposure to low doses of cypermethrin have demonstrated that placental transfer of pyrethroids results in its accumulation to the level that is sufficient to induce overexpression of cerebral and hepatic CYPs, persisting up to adulthood, in the exposed offsprings and produce alterations in the neurobehavioral activity in the exposed offsprings. The alterations in the circulating concentrations of hormones has demonstrated that due to endocrine disrupting potential, pyrethroids may permanently modify or imprint the expression of CYPs in the exposed offsprings. The imprinting of CYPs could be of toxicological significance as these low levels of pyrethroids are often encountered in the fields as well as in urban households to which pregnant women and developing children could be exposed [35].

Acknowledgments The authors are grateful to the director of CSIR-Indian Institute of Toxicology Research, Lucknow for his keen interest and support in carrying out the study. AS is thankful to CSIR, N. Delhi for providing Senior Research Fellowship. The financial assistance of Department of Biotechnology, N. Delhi is also gratefully acknowledged. IITR Communication No.3058

Competing financial declaration The authors declare that they have no competing financial interests.

References

1. Ray DE, Fry JR (2006) A reassessment of the neurotoxicity of pyrethroid insecticides. *Pharmacol Ther* 111(1):174–193
2. Breckenridge CB, Holden L, Sturgess N, Weiner M, Sheets L, Sargent D, Soderlund DM, Choi JS, Symington S, Clark JM, Burr S, Ray D (2009) Evidence for a separate mechanism of toxicity for the type I and the type II pyrethroid insecticides. *Neurotoxicology* 30(1):17–31
3. Rickard J, Brodie ME (1985) Correlation of blood and brain levels of the neurotoxic pyrethroids deltamethrin with the onset of symptoms in rats. *Pestic Biochem Physiol* 23:143–146
4. Ray DE, Forshaw PJ (2000) Pyrethroid insecticides: poisoning syndromes, synergies, and therapy. *J Toxicol Clin Toxicol* 38(2):95–101
5. Sheets LP, Doherty JD, Law MW, Reiter LW, Crofton KM (1994) Age-dependent differences in the susceptibility of rats to deltamethrin. *Toxicol Appl Pharmacol* 126(1):186–190
6. Anand SS, Bruckner JV, Haines WT, Muralidhara S, Fisher JW, Padilla S (2006) Characterization of deltamethrin metabolism by rat plasma and liver microsomes. *Toxicol Appl Pharmacol* 212(2):156–166
7. Anand SS, Kim KB, Padilla S, Muralidhara S, Kim HJ, Fisher JW, Bruckner JV (2006) Ontogeny of hepatic and plasma metabolism of deltamethrin in vitro: role in age-dependent acute neurotoxicity. *Drug Metab Dispos* 34(3):389–397
8. Dayal M, Parmar D, Dhawan A, Ali M, Dwivedi UN, Seth PK (2003) Effect of pretreatment of cytochrome P450 (P450) modifiers on neurobehavioral toxicity induced by deltamethrin. *Food Chem Toxicol* 41(3):431–437
9. DeMicco A, Cooper KR, Richardson JR, White LA (2010) Developmental neurotoxicity of pyrethroid insecticides in zebrafish embryos. *Toxicol Sci* 113(1):177–186
10. Shafer TJ, Meyer DA, Crofton KM (2005) Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environ Health Perspect* 113(2):123–136
11. Malaviya M, Husain R, Seth PK, Husain R (1993) Perinatal effects of two pyrethroid insecticides on brain neurotransmitter function in the neonatal rat. *Vet Hum Toxicol* 35(2):119–122
12. Tayebati SK, Di Tullio MA, Ricci A, Amenta F (2009) Influence of dermal exposure to the pyrethroid insecticide deltamethrin on rat brain microanatomy and cholinergic/dopaminergic neurochemistry. *Brain Res* 1301:180–188
13. Johri A, Dhawan A, Lakhani Singh R, Parmar D (2006) Effect of prenatal exposure of deltamethrin on the ontogeny of xenobiotic metabolizing cytochrome P450s in the brain and liver of offsprings. *Toxicol Appl Pharmacol* 214(3):279–289
14. Johri A, Yadav S, Singh RL, Dhawan A, Ali M, Parmar D (2006) Long lasting effects of prenatal exposure to deltamethrin on cerebral and hepatic cytochrome P450s and behavioral activity in rat offspring. *Eur J Pharmacol* 544(1–3):58–68
15. Elbetieha A, Da's S, Khamas W, Darmani H (2001) Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. *Arch Environ Contam Toxicol* 41(4):522–528
16. Parmar D, Dhawan A, Dayal M, Seth PK (1998) Immunochemical and biochemical evidence for expression of phenobarbital and 3-methylcholanthrene inducible isozymes of cytochrome P450 in rat brain. *Int J Toxicol* 1(17):619–630
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ et al (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193(1):265–275
18. Yadav S, Dhawan A, Singh RL, Seth PK, Parmar D (2006) Expression of constitutive and inducible cytochrome P450 2E1 in rat brain. *Mol Cell Biochem* 286(1–2):171–180

19. Shah PP, Saurabh K, Pant MC, Mathur N, Parmar D (2009) Evidence for increased cytochrome P450 1A1 expression in blood lymphocytes of lung cancer patients. *Mutat Res* 670(1–2):74–78
20. Baldwin SJ, Bramhall JL, Ashby CA, Yue L, Murdock PR, Hood SR, Ayrton AD, Clarke SE (2006) Cytochrome P450 gene induction in rats ex vivo assessed by quantitative real-time reverse transcriptase-polymerase chain reaction (TaqMan). *Drug Metab Dispos* 34(6):1063–1069
21. Wielgomas B, Krechniak J (2007) Toxicokinetic Interactions of α -cypermethrin and chlorpyrifos in Rats. *Polish J of Environ Stud* 16(2):267–274
22. Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, Yayoi O, Sato S, Nakabayashi K, Hata K, Sotomaru Y, Suzuki Y, Kono T (2012) Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. *PLoS Genet* 8(1):10024–10040
23. Miksys SL, Tyndale RF (2002) Drug-metabolizing cytochrome P450s in the brain. *J Psychiatry Neurosci* 27(6):406–415
24. Kaneko H, Izumi T, Ueda Y, Matsuo M, Miyamoto J (1984) Metabolism and placental transfer of stereoisomers of tetramethrin isomers in pregnant rats. *J Pestic Sci* 9:249–258
25. Shiba K, Kaneko H, Kakuta N, Yoshitake A, Miyamoto J (1990) Placental transfer of esfenvalerate and fenvalerate in pregnant rats. *J Pestic Sci* 15:169–174
26. Greally JM (2002) Short interspersed transposable elements (SINEs) are excluded from imprinted regions in the human genome. *Proc Natl Acad Sci* 99(1):327–332
27. Ke X, Thomas NS, Robinson DO, Collins A (2002) A novel approach for identifying candidate imprinted genes through sequence analysis of imprinted and control genes. *Hum Genet* 111(6):511–520
28. Daura-Oller E, Cabre M, Montero MA, Paternain JL, Romeu A (2009) A first-stage approximation to identify new imprinted genes through sequence analysis of its coding regions. *Comp Funct Genomics* 2009:549387
29. Nissbrandt H, Bergquist F, Jonason J, Engberg G (2001) Inhibition of cytochrome P450 2E1 induces an increase in extracellular dopamine in rat substantia nigra: a new metabolic pathway? *Synapse* 40(4):294–301
30. Shahabi HN, Andersson DR, Nissbrandt H (2008) Cytochrome P450 2E1 in the substantia nigra: relevance for dopaminergic neurotransmission and free radical production. *Synapse* 62(5):379–388
31. Singh AK, Tiwari MN, Upadhyay G, Patel DK, Singh D, Prakash O, Singh MP (2012) Long term exposure to cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult rats: postnatal exposure enhances the susceptibility during adulthood. *Neurobiol Aging* 33(2):404–415
32. Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, Weiner ML (2002) Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* 171(1):3–59
33. Roberge C, Beaudet MJ, Anderson A (2004) GABA(A)/central benzodiazepine receptor and peripheral benzodiazepine receptor ligands as inducers of phenobarbital-inducible CYP2B and CYP3A. *Biochem Pharmacol* 68(7):1383–1389
34. Konstandi M, Kostakis D, Harkitis P, Johnson EO, Marselos M, Adamidis K, Lang MA (2006) Benzo(alpha)pyrene-induced up-regulation of CYP1A2 gene expression: role of adrenoceptor-linked signaling pathways. *Life Sci* 79(4):331–341
35. Quiros-Alcala L, Bradman A, Nishioka M, Harnly ME, Hubbard A, McKone TE, Ferber J, Eskenazi B (2011) Pesticides in house dust from urban and farmworker households in California: an observational measurement study. *Environ Health* 10:19