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Ahr and Cyp1a2 genotypes both affect susceptibility to motor deficits following gestational and lactational exposure to polychlorinated biphenyls

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Abstract

Polychlorinated biphenyls (PCBs) are persistent organic pollutants known to cause adverse health effects and linked to neurological deficits in both human and animal studies. Children born to exposed mothers are at highest risk of learning and memory and motor deficits. We developed a mouse model that mimics human variation in the aryl hydrocarbon receptor and cytochrome P450 1A2 (CYP1A2) to determine if genetic variation increases susceptibility to developmental PCB exposure. In our previous studies, we found that high-affinity *Ahr^bCyp1a2(–/–)* and poor-affinity *Ahr^dCyp1a2(–/–)* knockout mice were most susceptible to learning and memory deficits following developmental PCB exposure compared with *Ahr^bCyp1a2(+/+)* wild type mice (C57BL/6J strain). Our follow-up studies focused on motor deficits, because human studies have identified PCBs as a potential risk factor for Parkinson's disease. Dams were treated with an environmentally relevant PCB mixture at gestational day 10 and postnatal day 5. We used a motor battery that included tests of nigrostriatal function as well as cerebellar function, because PCBs deplete thyroid hormone, which is essential to normal cerebellar development. There was a significant effect of PCB treatment in the rotarod test with impaired performance in all three genotypes, but decreased motor learning as well in the two *Cyp1a2(–/–)* knockout lines. Interestingly, we found a main

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5. Conflicts of interest.

The authors report no conflicts of interest.

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effect of genotype with corn oil-treated control *Cyp1a2*($-/-$) mice performing significantly worse than *Cyp1a2*($+/+$) wild type mice. In contrast, we found that PCB-treated high-affinity *Ahr^b* mice were most susceptible to disruption of nigrostriatal function with the greatest deficits in *Ahr^bCyp1a2*($-/-$) mice. We conclude that differences in AHR affinity combined with the absence of CYP1A2 protein affect susceptibility to motor deficits following developmental PCB exposure.

Keywords

Polychlorinated biphenyls; aryl hydrocarbon receptor; CYP1A2; nigrostriatal pathways; motor function; cerebellum

1. Introduction

Polychlorinated biphenyls (PCBs) are widespread persistent organic pollutants linked to numerous human health problems, with the most serious effects seen in children of exposed mothers (Ross 2004; Schantz et al., 2003; Jacobson et al., 2003). They are number 5 on the U.S. government's list of priority pollutants (ATSDR 2015). Worldwide, an estimated 200 billion kg remain in the environment (WHO 2003). The primary route of exposure is contaminated food, especially fatty fish, meat and dairy products (Malisch and Kotz, 2014; Langer et al., 2007; Gomara et al., 2005). New sources of PCB exposure have been reported with the inadvertent production of highly toxic PCB congeners (e.g. PCB 77 and PCB 153) during the synthesis of paint pigments (Anezaki et al., 2015; Hu & Hornbuckle, 2010) and the discovery of airborne PCBs near rural and urban schools (Marek et al., 2017). PCBs will remain a problem for generations because highly exposed cohorts are now reaching reproductive age (Bányiová et al., 2017; Quinn et al., 2011).

Multiple human studies found deficits in motor function in children exposed to high levels of PCBs (Boucher et al., 2016; Wilhelm et al., 2008; Vreugdenhil et al., 2002; Stewart et al., 2000). The hydroxylated metabolite 4-OH-CB 107 can also cause motor deficits in highly exposed children (Berghuis et al., 2013). In adults, an increased risk of Parkinson's disease (PD) was reported in women with high workplace exposures (Steenland et al., 2006) and in adults who consumed contaminated whale meat and blubber (Petersen et al., 2008). Rodent studies found adverse PCB effects in both the striatum (Caudle et al., 2006; Chishti et al., 1996) and cerebellum (Nguon et al., 2005). PCB effects on dopamine, the major neurotransmitter associated with motor function, are well known (Seegal et al., 1986, 1994, 1997, 2005).

PCBs occur as mixtures of coplanar congeners which can bind and activate the aryl hydrocarbon receptor and non-coplanar congeners which do not. Human studies clearly show differential responses to PCBs and related AHR agonists (Marek et al., 2014; Novotna et al., 2007; van Duursen et al., 2005; Tsuchiya et al., 2003). The AHR regulates three members of the cytochrome P450 family: CYP1A1, CYP1A2 and CYP1B1. The level of CYP1A2 found in human livers varies about 60-fold (Nebert & Dalton 2006), and maternal CYP1A2 can sequester planar pollutants to prevent transfer to offspring (Curran et al., 2011a; Dragin et al., 2006). In humans, there is a greater than 12-fold difference in the

inducibility of CYP1A1, although the polymorphism responsible has not been identified (Nebert 2017; Nebert et al. 2013).

We developed a mouse model to mimic human variation in the AHR and CYP1A2 to better understand genetic susceptibility to PCBs and similar pollutants. High-affinity *Ahr^b* mice will respond to low levels of xenobiotics such as dioxin and coplanar PCBs whereas poor-affinity *Ahr^d* mice are considered non-responders. To model the wide variation in CYP1A2, we used wild type *Cyp1a2*(+/+) mice and *Cyp1a2*(-/-) knockout mice (Denison & Faber 2017; Nebert 2017; Nebert & Dalton, 2006). We previously showed that both high-affinity *Ahr^bCyp1a2*(-/-) knockout mice and poor-affinity *Ahr^dCyp1a2*(-/-) mice were more susceptible to learning and memory deficits when exposed to an environmentally relevant mixture of PCBs during gestation and lactation compared with *Ahr^bCyp1a2*(+/+) wild type mice (Curran *et al.* 2011a-b, 2012). The studies described here extend those findings by testing the hypothesis that there is similar genetic susceptibility to PCB-induced motor deficits. Our motor battery was also designed to help clarify if PCBs affect motor function by disruption of nigrostriatal pathways or primarily target the cerebellum.

2. Materials and Methods 2.1 Animals

Three genotypes of mice were included. High-affinity *Ahr^bCyp1a2*(+/+) wild type mice were purchased from The Jackson Laboratory (Bar Harbor, ME) as C57BL/6J mice, which was the background strain for the two knockout lines used: *Ahr^bCyp1a2*(-/-) and poor-affinity *Ahr^dCyp1a2*(-/-). All animals were housed in standard shoebox polysulfone cages with corn cob bedding and one 5.1 cm² nestlet per week as enrichment. Water and Lab Diet 5015 chow were provided *ad libitum*.

Animals were kept on a 12h/12h light-dark cycle with all experiments conducted during the light cycle. Genotype was confirmed at the end of the behavior experiments. All experiments were approved by the Northern Kentucky University Institutional Animal Care and Use Committee. All husbandry and handling was in accordance with the Eighth Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines.

2.2 Breeding

Nulliparous females between 2.5 and 4 months of age were mated on a four-day breeding cycle with males of the same genotype. Females were separated from males the morning when a vaginal plug was found. Litters were culled or cross-fostered to balance litter size at 6 pups per dam, matching pups with dams of the same genotype and treatment. Pups were weaned at postnatal day 25, group housed by genotype, sex and treatment, and behavioral testing began at P60.

2.3 Treatments

We used the same dosing regimen (Table 1) described in our prior studies (Curran *et al.* 2011a,b), which was based on the toxic equivalency factors for coplanar PCBs 77, 126 and 169. Noncoplanar (PCB 105, 118, 138, 153, 180) congeners were selected based on reports of their high toxicity and prevalence in the human food supply (Van den berg *et al.*, 2006; Costabeber *et al.*, 2006; Langer *et al.*, 2007). Controls were treated with the corn oil vehicle

(Kroger, unstripped). Dams were randomly assigned to treatment groups and treated by gavage at a volume of 10µl/g body weight. at gestational day 10 (GD 10) and postnatal day 5 (PND 5). The PCB doses used in the current study were higher than typical human exposures, but consistent with risk assessment principles requiring higher doses of a toxicant to observe toxicity in the short timeframe of a typical animal study (Doull 2003) and at the low end of cumulative exposures used in other rodent studies of PCB neurotoxicity (Lein et al. 2007; Lee et al. 2011).

2.4 Chemicals

Polychlorinated biphenyl congeners were ordered from UltraScientific (N. Kingstown, RI). Unless otherwise noted, all other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

2.5 Western blot

CYP1A1 induction was confirmed in high-affinity *Ahr^b* mice using livers collected from P30 littermates of animals used in behavior. The liver was removed, rinsed in ice-cold phosphate buffered saline, blotted and stored at -80°C until processing. Approximately 500 mg of tissue per animal was homogenized using a polytron homogenizer and a buffer of 0.25 M sucrose, 10 mM HEPES, 1 mM Na₂EDTA, and 1 mM EGTA with 0.1% bovine serum albumin (BSA). The buffer was adjusted to pH 7.2 using KOH. Microsomes were prepared using multiple centrifugations to remove cellular debris and other organelles before ultracentrifugation at 40,000 g for 40 min. Microsomes were resuspended in 1 ml of the homogenization buffer. Protein concentrations were determined by the Bradford assay (Sigma-Aldrich, St. Louis MO), following the manufacturer's protocol. Microsomal proteins (10 µg/lane) were separated on 12% mixed alcohol-detergent-polyacrylamide gel electrophoresis (MAD-PAGE) under denaturing conditions (Brown, 1988). Separated proteins were transferred to PVDF membranes. Western blot analysis was performed using rabbit polyclonal anti-CYP1A1 antibody (Millipore AB1247) and a horseradish peroxidase-conjugated secondary antibody (Daiichi). The SuperSignal Pico enhanced chemiluminescence system (Pierce) was to detect primary antibody binding, with exposure times ranging from 10 to 60 sec. N = 4–6 per group.

2.6 EROD assay

Microsomes from liver, cortex and cerebellum were prepared as described in the previous section to measure CYP1A1 enzymatic activity in PCB-exposed and control animals using the well-known ethoxyresorufin-O-deethylase (EROD) assay. Unknowns were quantified using a standard curve, and purified human CYP1A1 was used as a positive control following the methods of Thompson et al. (2010). N = 5–9 per group.

2.7 Glutathione assay

Reduced glutathione (GSH) and oxidized GSSG were measured in liver, cortex and cerebellum using a standard kit (Cayman Chemicals, Ann Arbor MI) and following the manufacturer's protocols N = 3–5 per group.

2.8 Motor function tests

One male and one female from each litter were randomly assigned to behavioral testing. A comprehensive battery of tests was used to assess function in both the cerebellum and nigrostriatal pathways. Each animal went through the same experimental protocol, and each animal was limited to one test per day. Experiments were conducted within a 4 h time block to avoid confounding by circadian rhythms. Experiments are described in the order in which they were performed. Animal handlers and those analyzing the data were naïve to the genotype and treatment. N = 16 litters per group (Table 2) Video demonstrating the techniques and equipment can be viewed at: <https://www.youtube.com/watch?v=TximAxZcomk>

2.8.1 Rotarod—Mice were acclimated to the rotarod apparatus for one day at 0 rpm and one day at a constant 2 rpm speed. Testing was conducted with the rotarod set to accelerate from 1–20 rpm over 180 s, for a maximum trial of 300 s. Latency to fall was recorded. Mice received 3 trials per day for 5 days with an inter-trial interval of 5 min. Rotarod is one of the most widely used tests of cerebellar function (Nadler et al. 2006).

2.8.2 Gait analysis—The hind paws of mice were coated with nontoxic paint, then the mice walked down a 5 cm x 28 cm alley into a black-lined escape cage. Two days were used for training followed by a test day with three trials per day. Trials where mice ran or stopped were not included in the analysis. Stride length and stride width were measured, and the differential between the longest and shortest strides was calculated during analysis. Deficits in hind limb control result in uneven gait patterns in rodents (Fleming et al., 2004). Striatal lesions would result in more steps and shorter steps. Cerebellar lesions would result in unequal stride lengths. No mouse received more than 6 trials over 2 days before completing the task successfully.

2.8.3 Sticker removal—The sticker removal test was used to assess sensorimotor function (Schallert, 1988). Mice must detect the presence of a round 6.35 mm sticker on their snout and use their front paws to remove it. The latency to remove the sticker was recorded. Each mouse received a single test unless the sticker fell off or was not correctly placed on the snout. Mice not removing the sticker after 60 s were assigned a time of 60 s. No mouse received more than 3 trials.

2.8.4 Pole test—The pole test measures gross motor coordination and can detect impairments in nigrostriatal pathways (, Fernagut et al., 2003; Sedelis et al. 2001), which can be reversed by treatment with L-DOPA (Matsuura et al., 1997; Ogawa et al. 1985, 1987). A 50cm vertical pole of 8 mm diameter was placed inside the animal's home cage. Each mouse was placed at the top of the pole, with its head facing upward. Mice were trained to turn and climb down to the home cage for 2 days and tested on the Day 3. The time to turn and time to descend were recorded. Each mouse received 5 trials per day with a 60 s inter-trial interval.

2.8.5 Challenging balance beam—Mice were trained for two days (5 trials per day) on a smooth beam decreasing in width from 35 to 5cm. Each beam segment was 25 cm in

length for a total length of 1 m. Mice were tested on the third day with the beam covered by a wire mesh. Latency to cross, steps and slips were recorded, and the slip:step ratio was calculated during analysis. This protocol has been successfully used to assess nigrostriatal deficits in alpha-synuclein over-expressing mice and other PD mouse models. (Schultheis 2013, Fleming et al. 2004).

2.9 Data analysis

Behavior and EROD data were analyzed using SAS Proc Mixed Models Analysis of Variance with litter as the unit of analysis. We examined the main effects of treatment, genotype, and sex and their interactions. For rotarod, day was included as a repeated measure. When differences were found, we examined simple effects using the SAS “Slice” command with a correction for multiple post-hoc analyses. Measurements of reduced glutathione and oxidized GSSG were compared against control mice using the Holm-Sidak method. Data are presented as least square means $\pm$ the standard error of the mean (SEM). Western blot data is simply reported as CYP1A1 present or not, indicating whether or not AHR activation occurred.

3. Results

3.1 Assessment of AHR activation by CYP1A1 upregulation

Our Western blot and EROD results confirm that the AHR is only activated in PCB-treated high-affinity *Ahr^b* mice and not in poor-affinity *Ahr^d* mice or the corn oil-treated controls. At P30, levels of CYP1A1 protein were highest in livers of PCB-treated *Ahr^bCyp1a2(-/-)* knockout mice, but CYP1A1 protein was also present in PCB-treated *Ahr^bCyp1a2(+/+)* wild type mice (Fig 1). The EROD assays showed significantly higher CYP1A1 activity in the livers of P30 *Ahr^b* mice ($P < 0.001$) with nearly undetectable activity in PCB-treated *Ahr^dCyp1a2(-/-)* knockout mice and the corn oil-treated controls (Fig 2A). There was a trend for higher EROD activity in the cerebellum of *Ahr^bCyp1a2(-/-)* knockout mice ($P = 0.08$) compared with all other groups (Fig 2B). Similar trends ($P = 0.06$) were seen in cortex (data not shown).

3.2 Assessment of oxidative stress

Glutathione levels were significantly higher ($F_{2,11} = 8.48$; $P < 0.05$) in the liver of PCB-treated *Ahr^bCyp1a2(-/-)* knockout mice as well as levels of oxidized glutathione (GSSG) ($F_{2,11} = 16.75$; $P < 0.01$), indicating that oxidative stress response had been induced in these animals. There were no differences by treatment or genotype for GSH levels in the cortex or cerebellum; however PCB-treated *Ahr^bCyp1a2(-/-)* knockout mice had significantly higher levels of GSSG in the cortex ($F_{2,11} = 9.97$; $P < 0.05$) (Table 3)

3.3 Motor function test results

Data from behavior experiments are presented in the order in which they were performed.

3.3.1 Rotarod results—There was a significant main effect of genotype with both groups of *Cyp1a2(-/-)* mice having shorter latencies to fall compared with wild type mice ($F_{2,205} = 14.74$; $P < 0.0001$) and a significant main effect of treatment with PCB-treated mice having

shorter latencies to fall ($F_{2,205} = 16.81$; $P < 0.0001$). All groups of mice did show motor learning over the 5 days of testing with a significant main effect of day ($F_{4,587} = 75.83$; $P < 0.0001$); however, there was a significant gene x day interaction with *Ahr^bCyp1a2(+/+)* wild type mice showing significantly greater improvements over the 5 days of testing ($F_{8,656} = 4.26$; $P < 0.0001$). Female knockouts also showed the greatest impairments compared with controls (Figs. 3A–D).

3.3.2 Gait analysis—We found a significant main effect of genotype with both groups of *Cyp1a2(–/–)* mice having longer strides compared with wild type mice ($F_{2,248} = 16.95$; $P < 0.0001$) and a significant gene x treatment interaction ($F_{2,248} = 5.67$; $P < 0.01$). Both lines of *Cyp1a2(–/–)* knockout mice had longer strides than wild type mice, and PCB-treated *Ahr^bCyp1a2(+/+)* wild type mice had shorter stride lengths than all other groups (Fig. 4A–B). There was also a significant main effect of genotype for stride width (Fig. 4C) with *Ahr^bCyp1a2(–/–)* knockout mice having significantly narrower strides ($F_{2,248} = 10.48$; $P < 0.001$). There were no significant differences by genotype or treatment for stride differential ($P > 0.05$).

3.3.3 Sticker removal—PCB-treated *Ahr^bCyp1a2(–/–)* knockout mice had the longest latencies to remove the adhesive sticker; however, the differences were not statistically significant ($F_{2,281} = 1.37$; $P = 0.26$). There was a trend for a genotype effect ($F_{2,281} = 2.80$; $P = 0.062$) with *Ahr^bCyp1a2(–/–)* knockout mice having the longest latencies compared with wild type mice and *Ahr^dCyp1a2(–/–)* knockout mice (Fig. 5).

3.3.4 Pole test—There were no differences in the time to turn or the time to descend the pole based on treatment; however, females had significantly shorter turn ($F_{1,295} = 5.56$; $P < 0.05$) and descent times compared with males ($F_{1,295} = 4.74$; $P < 0.05$), and *Ahr^dCyp1a2(–/–)* knockout mice had significantly shorter turn ($F_{2,295} = 4.27$; $P < 0.05$) and descent times ($F_{2,295} = 7.23$; $P < 0.001$) compared with *Ahr^b* knockout and wild type mice (Figs. 6A–B).

3.3.5 Challenging balance beam—There was a significant main effect of genotype for latency to cross the balance beam with *Ahr^dCyp1a2(–/–)* knockout mice having significantly shorter latencies ($F_{2,121} = 3.36$; $P < 0.05$) compared with *Ahr^b* knockout and wild type mice (Fig. 7A). There was a significant gene x treatment interaction ($F_{2,117} = 6.54$; $P < 0.01$) for the number of slips with PCB-treated *Ahr^b* mice having more slips while PCB-treated *Ahr^dCyp1a2(–/–)* knockout mice had significantly fewer slips (Fig. 7B). There was also a significant gene x treatment interaction ($F_{2,117} = 6.07$; $P < 0.01$) for the ratio of slips/steps with PCB-treated *Ahr^b* mice having more slips per step while PCB-treated *Ahr^dCyp1a2(–/–)* knockout mice had significantly fewer slips per step (Fig. 7C).

4. Discussion and conclusions

Our data confirm that an environmentally relevant PCB mixture only activates the AHR in high-affinity *Ahr^b* mice and that *Cyp1a2(+/+)* wild type mice show fewer motor impairments following developmental PCB exposure. This supports our previous findings (Curran et al. 2011b, 2012) and extends them to motor deficits. We did not find evidence of significant oxidative stress following PCB exposure, although it appears that the antioxidant

response system had been activated, since both oxidized and reduced glutathione levels were significantly increased in the most susceptible *Ahr^bCyp1a2(-/-)* mice (Table 3).

Our motor battery was designed to assess both nigrostriatal pathways and cerebellar function, and the data are consistent with impairments in those brain regions. PCB-treated mice from all three genotypes showed impaired performance on the rotarod compared to corn oil-treated controls, suggesting a cerebellar deficit. Motor learning was reduced in the two knockout lines compared with wild type mice. Interestingly, there was also a motor deficit in both corn oil-treated lines of *Cyp1a2(-/-)* mice. There is evidence to suggest CYP1A2 has a normal function in the brain. CYP1A2 is differentially regulated in the cortex and cerebellum (Iba et al., 2003). The enzyme can also be induced in the brain in a region-specific manner with highest levels seen in the pons, medulla, cerebellum, frontal cortex (Yadav et al., 2006) and hypothalamus (Korkalainen et al., 2005). CYP1A2 is normally down-regulated during cerebellar granule cell migration (Mulero-Navarro et al. 2003). Together with our findings, these data warrant further study of CYP1A2's role in cerebellar development and function.

Results from the gait analysis did not support our hypothesis of genetic susceptibility in *Cyp1a2(-/-)* mice. In contrast, PCB-treated *Ahr^bCyp1a2(+/+)* mice had shorter strides than corn oil-treated control mice and both groups of PCB-treated knockout mice. Nigrostriatal lesions result in shorter strides (Fleming et al. 2004), so this suggests some impairment in the PCB-treated wild type mice. We previously reported striatal dopamine levels were significantly lower in PCB-treated *Ahr^bCyp1a2(-/-)* mice compared with PCB-treated *Ahr^bCyp1a2(+/+)* mice ($p < 0.01$) (Curran et al. 2011a), so it's unlikely this impairment was caused by an absolute loss of dopamine.

Our findings are consistent with Caudle et al. (2006) who reported a significant decrease in expression of the dopamine transporter (DAT) in PCB-treated C57BL/6J mice, but no difference in striatal dopamine levels. Bemis and Seegal (2004) reported that PCB mixtures inhibit the vesicular monoamine transporter (VMAT) in synaptosomes from adult Long-Evans rats. Interestingly, Akahoshi et al. (2009, 2012) demonstrated that the liganded *AHR* upregulates tyrosine hydroxylase, the rate-limiting enzyme in dopamine production. Together, this suggests future work is needed to examine the inter-related effects of PCB mixtures on dopamine production, metabolism and transport.

The major finding in the pole test was a shorter latency to turn and descend the pole by *Ahr^dCyp1a2(-/-)* knockout mice. This difference could result from increased anxiety and greater motivation to return to the home cage or hyperactivity which has also been reported in PCB neurotoxicity studies. Our prior studies with poor affinity *Ahr^dCyp1a2(+/+)* and *Ahr^dCyp1a2(-/-)* mice found no evidence of hyperactivity in open field locomotor testing, fewer zone crossings in the elevated zero maze (Curran et al. 2011b) and a heightened startle response in *Ahr^dCyp1a2(-/-)* mice (Curran et al. 2012). This argues in favor of an anxiety-like phenotype in *Ahr^d* mice and against hyperactivity. Since latencies were shorter than control mice, the results cannot be interpreted as an impairment in motor function.

The challenging balance beam results support the hypothesis that *Ahr^dCyp1a2(-/-)* knockout mice have higher motivation to reach the goal box or home cage. Both PCB-treated and control mice from this line had significantly shorter latencies to traverse the beam compared with *Ahr^b* mice. Both PCB-treated *Ahr^b* groups showed a trend toward more slips, which can be an indication of an impairment in nigrostriatal function.

Efforts to identify a mode of action responsible for the distinct patterns of PCB-induced motor impairments observed in these experiments will need to consider both the genetic differences and the well-established model of noncoplanar PCB neurotoxicity mediated by the ryanodine receptor (RyR) and calcium dysregulation (Dingemans et al. 2016, Pessah et al. 2010, Roegge et al. 2006, Gafni et al. 2004). We note that our PCB mixture does contain noncoplanar PCBs, but the congeners included do not have the same high potency at the ryanodine receptor as its prototypical agonist PCB 95. Therefore, it is likely that an alternate mechanism or mechanisms are needed to explain all of the observed effects. In support of that concept, Roegge et al. (2006) found increased levels of RyR1 in PCB-treated Long-Evans rats, but no evidence of increased receptor binding when looking at changes in the cerebellum. Meanwhile, Nguon et al (2005) reported increased GFAP expression and reduced cerebellar mass in Sprague-Dawley rats exposed to Aroclor during development. Males had more severe motor impairments on rotarod and greater increases in GFAP compared with females. In contrast, we found greater impairments in female mice. Llansola et al. (2009) identified the glutamate–NO–cGMP pathway as a cerebellar target of both coplanar PCB 126 and noncoplanar PCB 153 in Wistar rats. A follow-up study confirmed motor deficits for both PCB126 and PCB 153 with younger males at higher risk (Cauli et al. 2013).

A limitation of our studies was the use of gavage dosing. Although this allows precise dosing, it would be important to repeat these experiments using a chronic, low-level exposure in food. This is commonly done in rat studies (Miller et al. 2017, Roegge et al. 2006, Widholm et al. 2004), but can be a challenge when using mice which consume much smaller quantities of food each day. In addition, longer-term studies would allow an assessment of motor function in aged mice, which would better match the typical course of human Parkinson's disease patients. Since behavioral testing began at P60, it is possible that only minor impairments would be seen in nigrostriatal pathways at that age with more pronounced effects in animals over 1 y of age.

Further work is needed to assess gene expression in the substantia nigra, striatum and cerebellum at multiple time points to verify that there were no adverse effects on dopaminergic neurons involved in motor function and to identify potential modes of action for the observed motor deficits. A histological examination of those tissues could also reveal morphological changes related to abnormal development. It would also be important to look at changes related to thyroid hormone depletion, because we previously found that circulating thyroxine (T4) levels were reduced 80% in *Ahr^bCyp1a2(-/-)* knockout mice compared with control levels at P6 (Curran et al. 2011a). Hydroxylated PCBs can cross the placenta (Meerts et al. 2002) and mimic the action of thyroid hormone (Giera et al. 2011, Darras et al. 2008). Recent work has implicated CYP1A1 in metabolism of non-coplanar PCB congeners (Wadzinski et al. 2014, Gauger et al. 2007) to thyromimetic forms. Both 4-

OH-PCB 107 (Berghuis et al. 2013) and OH-PCB 106 (Haijima et al. 2017) cause motor deficits, so it will be important to look into differential metabolism of parent congeners in the susceptible and resistant lines of mice.

4.1 Conclusions

Our data provides evidence that developmental exposure to PCBs lead to motor deficits consistent with adverse effects on cerebellar development and function as well as disruption of nigrostriatal pathways. The data presented here and our previous studies (Curran et al. 2011a and 2011b) provide strong evidence that allelic differences at the *Ahr* and *Cyp1a2* loci affect developmental neurotoxicity to polychlorinated biphenyls. We have extended our previous studies on learning and memory deficits to demonstrate that high-affinity *Ahr^bCyp1a2*($-/-$) knockout mice are uniquely susceptible to motor dysfunction following PCB exposure during gestation and lactation. By including corn oil-treated control mice of three genotypes in our studies, we also uncovered a previously unreported motor deficit in *Cyp1a2*($-/-$) mice, regardless of *Ahr* genotype. Given known human variation in the aryl hydrocarbon receptor and CYP1A2, these studies support the use of our mouse model to explore developmental neurotoxicity of similar persistent organic pollutants and the role of CYP1A2 in normal brain development and function.

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Highlights

- Genetic variation in the aryl hydrocarbon receptor and cytochrome P450 1A2 (CYP1A2) alter susceptibility to developmental PCB exposure.
- PCB-treated high-affinity Ahrbmice had greater deficits in motor function tests associated with nigrostriatal pathways compared with poor-affinity Ahrdmice.
- PCB-treated Cyp1a2(−/−) knockout mice were more susceptible to motor deficits on tests associated with cerebellar function.
- Unexpectedly, corn oil-treated control Cyp1a2(−/−) knockout mice also had deficits on the rotarod test compared with Cyp1a2(−/−) wild type mice.
- Our findings cannot rule out PCBs as a risk factor for Parkinson's disease, but our data indicate more than one brain region required for normal motor function are affected by developmental PCB exposure.

Fig. 1.

Western blot of CYP1A1 in liver. CYP1A1 is an inducible enzyme typically not detectable in tissues unless the aryl hydrocarbon (AHR) is activated. Our Western blot analysis confirmed the AHR was only activated in high-affinity *Ahr^b* mice compared with corn oil-treated controls and poor-affinity *Ahr^d* mice.

Fig. 2A

Fig 2B

Fig. 2.

Fig. 2A EROD activity in liver. There was a significant gene x treatment interaction with higher EROD activity in liver of *Ahr^b* mice compared with poor-affinity *Ahr^d* mice. N = 9 per group. *** P < 0.001

Fig 2B EROD activity in cerebellum. There was a trend for higher EROD activity in the cerebellum of *Ahr^bCyp1a2*(-/-) mice. P = 0.08. N = 5 per group.

Fig. 3A

Fig. 3B

Fig. 3C

Fig. 3D

Fig. 3.

Fig. 3A Rotarod performance by genotype. There was a main effect of genotype with *Cyp1a2(-/-)* having significantly shorter latencies to fall off the rotarod. *** $P < 0.001$.

Fig. 3B Rotarod performance by treatment. There was a main effect of treatment on rotarod performance with PCB-treated mice having significantly shorter latencies to fall off the rotarod. *** $P < 0.001$.

Fig. 3C Rotarod performance over time (motor learning). All groups of mice showed improvement over 5 days of testing, but high-affinity *Ahr^bCyp1a2(+/+)* showed greater motor learning compared with *Cyp1a2(-/-)* knockout mice with a significant gene x day interaction. $P < 0.001$.

Fig. 3D. Sex differences in rotarod performance. Female mice with the *Cyp1a2*(-/-) genotype showed the greatest impairments on the rotarod test compared with *Cyp1a2*(+/+) wild type mice. *P < 0.05, ** P < 0.01, *** P < 0.001.

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Fig. 4.

A. Gait stride length. *Ahr^bCyp1a2(+/+)* wild type mice had significantly shorter strides than all other groups. ** $P < .001$. B. Gait stride length. PCB-treated *Ahr^bCyp1a2(+/+)* wild type mice had significantly shorter strides than all other groups. ** $P < .01$. C. Gait stride width. *Ahr^bCyp1a2(-/-)* mice had significantly narrower strides compared with the other two genotypes, but there was no effect of PCB treatment. *** $P < .001$.

Fig. 5. Latency to remove adhesive sticker

There was a trend for a gene x treatment interaction with PCB-treated *Ahr^bCyp1a2(-/-)* mice having the longest latencies of all groups. $P = 0.06$.

Fig. 6A

Fig. 6B

Fig. 6.**Fig. 6A** Pole turn time.

There was a main effect of genotype, but no effect of PCB treatment with poor-affinity *Ahr^DCyp1a2(-/-)* mice having the shortest latencies to turn downward on the pole. * $P < 0.05$.

Fig. 6B Pole descent time. There was a main effect of genotype, but no effect of PCB treatment with poor-affinity *Ahr^DCyp1a2(-/-)* having the shortest latencies to descend the pole back to the home cage. ** $P < 0.01$.

Fig. 7A

Fig. 7B

Fig. 7C

Fig. 7.

Fig. 7A. Challenging beam latency. *Ahr^dCyp1a2(-/-)* mice had the shortest latency to cross the balance beam, regardless of treatment. * $P < 0.05$

Fig. 7B Mean slips on challenging beam. There was a significant gene x treatment interaction with PCB-treated *Ahr^b* mice having more slips than corn oil-treated controls of the same genotype and poor-affinity *Ahr^d* mice having significantly fewer than corn oil-treated controls of the same genotype. $P < 0.1$, ** $P < 0.01$.

Fig. 7C Slip to Step ratio on challenging beam. There was a significant gene x treatment interaction with PCB-treated *Ahr^bCyp1a2(-/-)* mice having more slips per step than their corn oil-treated controls mice and poor-affinity *Ahr^dCyp1a2(-/-)* mice having significantly fewer slips per step than their corn oil-treated controls. ** $P < 0.01$.

Table 1

PCB congeners and concentrations used in dosing mixture.

PCB congener	Planarity	IUPAC #	Dose
2,3,3',4,4'-Pentachlorobiphenyl	non-coplanar	105	10 mg/kg
2,3',4,4',5-Pentachlorobiphenyl	non-coplanar	118	10 mg/kg
2,2',3,4,4',5'-Hexachlorobiphenyl	non-coplanar	138	10 mg/kg
2,2',4,4',5,5'-Hexachlorobiphenyl	non-coplanar	153	10 mg/kg
2,2',3,4,4',5,5'-Heptachlorobiphenyl	non-coplanar	180	10 mg/kg
3,3',4,4'-Tetrachlorobiphenyl	coplanar	77	5 mg/kg
3,3',4,4',5-Pentachlorobiphenyl	coplanar	126	25 µg/kg
3,3',4,4',5,5'-Hexachlorobiphenyl	coplanar	169	250 µg/kg

Table 2

Group sizes for behavioral experiments

Genotype	Treatment	N per group
<i>Ahr^bCyp1a2(+/+)</i>	Corn oil	16
<i>Ahr^bCyp1a2(+/+)</i>	PCB	19
<i>Ahr^bCyp1a2(-/-)</i>	Corn oil	16
<i>Ahr^bCyp1a2(-/-)</i>	PCB	17
<i>Ahr^dCyp1a2(-/-)</i>	Corn oil	21
<i>Ahr^dCyp1a2(-/-)</i>	PCB	16

Table 3

Measurements of reduced and oxidized glutathione in live and cortex. Bold indicates significant differences from control mice

<i>Genotype</i>	<i>Treatment</i>	<i>GSH-Liver</i>	<i>GSSG-Liver</i>	<i>GSSG-Cortex</i>
<i>AhrbCyp1a2(+/+)</i>	corn oil	51 ± 5.8	12.5 ± 0.5	6.0 ± 0.8
<i>AhrbCyp1a2(+/+)</i>	PCB	43.5 ± 4.5	11.3 ± 0.4	7.0 ± 0.6
<i>AhrbCyp1a2(-/-)</i>	PCB	64.8 ± 5.8	14.3 ± 0.5	9.7 ± 0.8
<i>AhrdCyp1a2(-/-)</i>	PCB	50.8 ± 6.9	12.8 ± 0.7	9.1 ± 1.0