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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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1 **Abstract**

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

21

22 **Keywords:** Polychlorinated biphenyls, aryl hydrocarbon receptor, CYP1A2, Parkinson's disease, motor
23 function, cerebellum

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24 **1. Introduction.**

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

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

43 PCBs occur as mixtures of coplanar congeners which can bind and activate the aryl hydrocarbon receptor
44 and non-coplanar congeners which do not. Human studies clearly show differential responses to PCBs and
45 related AHR agonists (Marek et al. 2014, Novotna *et al*. 2007, van Duursen *et al*. 2005, Tsuchiya et al.
46 2003). The AHR regulates three members of the cytochrome P450 family: CYP1A1, CYP1A2 and
47 CYP1B1.The level of CYP1A2 found in human livers varies about 60-fold (Nebert et al. 2006), and
48 maternal CYP1A2 can sequester planar pollutants to prevent transfer to offspring (Curran et al. 2011a,

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49 Dragin et al. 2006,). In humans, there is a greater than 12-fold difference in the inducibility of CYP1A1,
50 although the polymorphism responsible has not been identified (Nebert et al. 2004).

51 We developed a mouse model to mimic human variation in the AHR and CYP1A2 to better understand
52 genetic susceptibility to PCBs and similar pollutants. We showed that both high-affinity *Ahr*^{b}*Cyp1a2(-/-)*
53 knockout mice and poor-affinity *Ahr*^{d}*Cyp1a2(-/-)* mice were more susceptible to learning and memory
54 deficits when exposed to an environmentally relevant mixture of PCBs during gestation and lactation
55 compared with *Ahr*^{b}*Cyp1a2(+/+)* wild type mice (Curran *et a*l. 2011a-b, 2012). The studies described here
56 extend those findings by testing the hypothesis that there is similar genetic susceptibility to PCB-induced
57 motor deficits. Our motor battery was also designed to help clarify if PCBs are a significant risk factor for
58 Parkinson's disease.

59 **2. Materials and Methods**

60 **2.1 Animals.**

61 Three genotypes of mice were included. High-affinity *Ahr*^{b}*Cyp1a2(+/+)* wild type mice were purchased
62 from The Jackson Laboratory (Bar Harbor, ME) as C57BL/6J mice, which was the background strain for
63 the two knockout lines used: *Ahr*^{b}*Cyp1a2(-/-)* and poor-affinity *Ahr*^{d}*Cyp1a2(-/-)*. All animals were housed
64 in standard shoebox polysulfone cages with corncob bedding and one 5.1 cm^2 nestlet per week as
65 enrichment. Water and Lab Diet 5015 chow were provided *ad libitum.*

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

71 **2.2 Breeding.**

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

77 **2.3 Treatments.**

78 We used an environmentally relevant mixture of coplanar (PCB 77, 126, and 169) and noncoplanar (PCB
79 105, 118, 138, 153, 180) congeners. Dams were randomly assigned to treatment groups and treated by
80 gavage at gestational day 10 (GD 10) and postnatal day 5 (PND 5). Controls were treated with the corn oil
81 vehicle. Additional details on dosing were reported in Curran et al. (2011a).

82 **2.4 Chemicals.**

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

85 **2.5 Western blot.**

86 CYP1A1 induction was confirmed in high-affinity *Ahr*^{b} mice using livers collected from P30 littermates
87 of animals used in behavior. The liver was removed, rinsed in ice-cold phosphate buffered saline, blotted
88 and stored at -80°C until processing. Approximately 500 mg of tissue per animal was homogenized using
89 a polytron homogenizer and a buffer of 0.25 M sucrose, 10 mM HEPES, 1 mM Na2EDTA, and 1 mM
90 EGTA with 0.1% bovine serum albumin (BSA). The buffer was adjusted to pH 7.2 using KOH.
91 Microsomes were prepared using multiple centrifugations to remove cellular debris and other organelles
92 before ultracentrifugation at 40,000 g for 40 min. Microsomes were resuspended in 1 ml of the
93 homogenization buffer. Protein concentrations were determined by the Bradford assay (Sigma-Aldrich,
94 St. Louis MO), following the manufacturer's protocol. Microsomal proteins (10 μg/lane) were separated
95 on 12% mixed alcohol-detergent-polyacrylamide gel electrophoresis (MAD–PAGE) under denaturing

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96 conditions (Brown, 1988). Separated proteins were transferred to PVDF membranes. Western blot
97 analysis was performed using rabbit polyclonal anti-CYP1A1 antibody (Millipore AB1247) and a
98 horseradish peroxidase-conjugated secondary antibody (Daiichi). The SuperSignal Pico enhanced
99 chemiluminescence system (Pierce) was to detect primary antibody binding, with exposure times ranging
100 from 10 to 60 sec. N = 4-6 per group.

101 **2.6 EROD assay.**

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

107 **2.7 Glutathione assay.**

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

111 **2.8 Motor function tests.**

112 One male and one female from each litter were randomly assigned to behavioral testing. A comprehensive
113 battery of tests was used to assess function in both the cerebellum and nigrostriatal pathways. Each
114 animal went through the same experimental protocol, and each animal was limited to one test per day.
115 Experiments were conducted within a 4 h time block to avoid confounding by circadian rhythms.
116 Experiments are described in the order in which they were performed. Animal handlers and those
117 analyzing the data were naïve to the genotype and treatment. N $\geq$ 15 litters per group. Video
118 demonstrating the techniques and equipment can be viewed at:
119 https://www.youtube.com/watch?v=TximAxZcomk

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120 **2.8.1 Rotarod**.

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

125 **2.8.2 Gait analysis.**

126 The hind paws of mice were coated with nontoxic paint, then the mice walked down a 5 cm x 28 cm alley
127 into a black-lined escape cage. Two days were used for training followed by a test day with three trials
128 per day. Trials where mice ran or stopped were not included in the analysis. Stride length and stride width
129 were measured, and the differential between the longest and shortest strides was calculated during
130 analysis. Deficits in hind limb control result in uneven gait patterns in rodents (Fleming et al. 2004).
131 Striatal lesions would result in more steps and shorter steps. Cerebellar lesions would result in unequal
132 stride lengths.

133 **2.8.3 Sticker removal.**

134 The sticker removal test was used to assess sensorimotor function (Schallert, 1988). Mice must detect the
135 presence of a round 6.35 mm sticker on their snout and use their front paws to remove it. The latency to
136 remove the sticker was recorded. Each mouse received a single test unless the sticker fell off or was not
137 correctly placed on the snout. Mice not removing the sticker after 60 s were assigned a time of 60 s.

138 **2.8.4 Pole test.**

139 The pole test measures gross motor coordination and can detect impairments in nigrostriatal pathways
140 (Sedelis et al. 2001, Fernagut et al., 2003), which can be reversed by treatment with L-DOPA (Ogawa et
141 al. 1985, 1987; Matsuura et al. 1997). A 50cm vertical pole was placed inside the animal's home cage.
142 Each mouse was placed at the top of the pole, with its head facing upward. Mice were trained to turn and

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143 climb down to the home cage with 5 trials per day for 2 days and tested on the Day 3. The time to turn
144 and time to descend were recorded.

145 **2.8.5 Challenging balance beam.**

146 Mice were trained for two days (5 trials per day) on a smooth beam decreasing in width from 35 to 5cm.
147 Each beam segment was 25 cm in length for a total length of 1 m. Mice were tested on the third day with
148 the beam covered by a wire mesh. Latency to cross, steps and slips were recorded, and the slip:step ratio
149 was calculated during analysis. This protocol has been successfully used to assess nigrostriatal deficits in
150 alpha-synuclein over-expressing mice and other PD mouse models. (Schultheis 2013, Fleming et al.
151 2004).

152 **2.9 Data analysis.**

153 Data were analyzed using SAS Proc Mixed Models Analysis of Variance with litter as the unit of
154 analysis. For rotarod, day was included as a repeated measure. When differences were found, we
155 examined slice effects with a correction for multiple post-hoc analyses. Data are presented as least square
156 means ± the standard error of the mean (SEM).

157 **3. Results**

158 **3.1 Assessment of AHR activation by CYP1A1 upregulation.**

159 Our Western blot and EROD results confirm that the AHR is only activated in PCB-treated high-affinity
160 Ahr^b mice and not in poor-affinity Ahr^d mice or the corn oil-treated controls. At P30, levels of CYP1A1
161 protein were highest in livers of PCB-treated *Ahr*^{b}*Cyp1a2(-/-)* knockout mice, but CYP1A1 protein was
162 also present in PCB-treated *Ahr*^{b}*Cyp1a2(+/+)* wild type mice (Fig 1). The EROD assays showed
163 significantly higher CYP1A1 activity in the livers of P30 Ahr^b mice ($P < 0.001$) with nearly undetectable
164 activity in PCB-treated *Ahr*^{d}*Cyp1a2(-/-)* knockout mice and the corn oil-treated controls (Fig 2A). There

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165 was a trend for higher EROD activity in the cerebellum of *Ahr^b Cyp1a2(-/-)* knockout mice (P = 0.08)
166 compared with all other groups (Fig 2B). Similar trends (P = 0.06) were seen in cortex (data not shown).

167 **3.2 Assessment of oxidative stress.**

168 Glutathione levels were significantly higher ($F_{2,11} = 8.48$; $P < 0.05$) in the liver of PCB-treated
169 *Ahr^b Cyp1a2(-/-)* knockout mice as well as levels of oxidized glutathione (GSSG) ($F_{2,11} = 16.75$; $P < 0.01$),
170 indicating that oxidative stress response had been induced in these animals. There were no differences by
171 treatment or genotype for GSH levels in the cortex or cerebellum; however PCB-treated *Ahr^b Cyp1a2(-/-)*
172 knockout mice had significantly higher levels of GSSG in the cortex ($F_{2,11} = 9.97$; $P < 0.05$).

173 **3.3 Motor function test results.**

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

175 **3.3.1 Rotarod results.**

176 There was a significant main effect of genotype with both groups of *Cyp1a2(-/-)* mice having shorter
177 latencies to fall compared with wild type mice ($F_{2,205} = 14.74$; $P < 0.0001$) and a significant main effect of
178 treatment with PCB-treated mice having shorter latencies to fall ($F_{2,205} = 16.81$; $P < 0.0001$). All groups of
179 mice did show motor learning over the 5 days of testing with a significant main effect of day ($F_{4,587} =$
180 75.83; $P < 0.0001$); however, both groups of knockouts showed less improvement compared with
181 *Ahr^b Cyp1a2(+/+)* wild type mice. Female knockouts also showed the greatest impairments compared
182 with controls (Figs. 3A-D).

183 **3.3.2 Gait analysis.**

184 We found a significant main effect of genotype with both groups of *Cyp1a2(-/-)* mice having longer
185 strides compared with wild type mice ($F_{2,248} = 16.95$; $P < 0.0001$) and a significant gene x treatment
186 interaction ($F_{2,248} = 5.67$; $P < 0.01$). Both lines of *Cyp1a2(-/-)* knockout mice had longer strides than wild
187 type mice, and PCB-treated *AhrbCyp1a2(+/+)* wild type mice had shorter stride lengths than all other

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188 groups (Fig. 4A). There was also a significant main effect of genotype for stride width (Fig. 4B) with
189 *Ahr*^{d}*Cyp1a2(-/-)* knockout mice having significantly wider strides (($F_{2,248}$ = 10.48; P < 0.001). There were
190 no significant differences by genotype or treatment for stride differential (P > 0.05).

191 **3.3.3 Sticker removal.**

192 PCB-treated *Ahr*^{b}*Cyp1a2(-/-)* knockout mice had the longest latencies to remove the adhesive sticker;
193 however, the differences were not statistically significant ($F_{2,281}$ = 1.37; P = 0.26). There was a trend for a
194 genotype effect ($F_{2,281}$ = 2.80; P = 0.062) with *Ahr*^{b}*Cyp1a2(-/-)* knockout mice having the longest latencies
195 compared with wild type mice and *Ahr*^{d}*Cyp1a2(-/-)* knockout mice (Fig. 5).

196 **3.3.4 Pole test.**

197 There were no differences in the time to turn or the time to descend the pole based on treatment; however,
198 females had significantly shorter turn ($F_{1,295}$ = 5.56; P <0.05) and descent times compared with males
199 ($F_{1,295}$ = 4.74; P <0.05), and *Ahr*^{d}*Cyp1a2(-/-)* knockout mice had significantly shorter turn ($F_{2,295}$ = 4.27; P
200 <0.05) and descent times ($F_{2,295}$ = 7.23; P <0.001) compared with *Ahr*^{b} knockout and wild type mice (Figs.
201 6A-B).

202 **3.3.5 Challenging balance beam.**

203 There was a significant main effect of genotype for latency to cross the balance beam with *Ahr*^{d}*Cyp1a2(-*
204 */-)* knockout mice having significantly shorter latencies ($F_{2,121}$ = 3.36; P <0.05) compared with *Ahr*^{b}
205 knockout and wild type mice (Fig. 7A). There was a significant gene x treatment interaction ($F_{2,117}$ = 6.54;
206 P <0.01) for the number of slips with PCB-treated *Ahr*^{b} mice having more slips while PCB-treated
207 *Ahr*^{d}*Cyp1a2(-/-)* knockout mice had significantly fewer slips (Fig. 7B). There was also a significant gene
208 x treatment interaction ($F_{2,117}$ = 6.07; P <0.01) for the ratio of slips/steps with PCB-treated *Ahr*^{b} mice
209 having more slips per step while PCB-treated *Ahr*^{d}*Cyp1a2(-/-)* knockout mice had significantly fewer
210 slips per step (Fig. 7C).

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211 **4. Discussion and conclusions.**

212 Our data confirm that an environmentally relevant PCB mixture only activates the AHR in high-affinity
213 *Ahr^b* mice and that CYP1A2 is protective against PCB-induced neurotoxocity. This supports our previous
214 findings (Curran et al. 2011b, 2012) and extends them to motor deficits. We did not find evidence of
215 significant oxidative stress following PCB exposure, although it appears that the antioxidant response
216 system had been activated, since both oxidized and reduced glutathione levels were significantly
217 increased in the most susceptible *Ahr^bCyp1a2(-/-)* mice.

218 Our motor battery was designed to assess both nigrostriatal pathways and cerebellar function, and the data
219 indicate that both regions are affected, but not equally. PCB-treated mice from all three genotypes showed
220 impaired performance on the rotarod compared to corn oil-treated controls, indicating a cerebellar deficit.
221 Motor learning was reduced in the two knockout lines. Interestingly, there was also a motor deficit in both
222 corn oil-treated lines of *Cyp1a2(-/-)* mice. There is evidence to suggest CYP1A2 has a normal function in
223 the brain. CYP1A2 is differentially regulated in the cortex and cerebellum (Iba et al. 2003). The enzyme
224 can also be induced in the brain in a region-specific manner with highest levels seen in the pons, medulla,
225 cerebellum, frontal cortex (Yadav et al. 2006) and hypothalamus (Korkalainen et al. 2005). CYP1A2 is
226 normally down-regulated during cerebellar granule cell migration (Mulero-Navarro et al. 2003). Together
227 with our findings, these data warrant further study of CYP1A2's role in cerebellar development and
228 function.

229 Results from the gait analysis did not support our hypothesis of genetic susceptibility in *Cyp1a2(-/-)* mice.
230 In contrast, PCB-treated *Ahr^bCyp1a2(+/+)* mice had shorter strides than corn oil-treated control mice and
231 both groups of PCB-treated knockout mice. Nigrostriatal lesions result in shorter strides (Fleming et al.
232 2004), so this suggests some impairment in the PCB-treated wild type mice. We previously reported
233 striatal dopamine levels were significantly lower in PCB-treated *Ahr^bCyp1a2(–/–)* mice compared with
234 PCB-treated *Ahr^bCyp1a2(+/+)* mice ($p < 0.01$), so it's unlikely this impairment was caused by an
235 absolute loss of dopamine.

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

243 The major finding in the pole test was a shorter latency to turn and descend the pole by Ahr^{d}*Cyp1a2(-/-)*
244 knockout mice; however, this difference could result from increased anxiety and greater motivation to
245 return to the home cage. Since latencies were shorter than control mice, the results cannot be interpreted
246 as an impairment in motor function. Similarly, Ahr^{d}*Cyp1a2(-/-)* knockout mice had the shortest latency to
247 remove an adhesive sticker, indicating a genetic difference in behavior, but not a PCB effect.

248 The challenging balance beam results support the hypothesis that Ahr^{d}*Cyp1a2(-/-)* knockout mice have
249 higher motivation to reach the goal box or home cage. Both PCB-treated and control mice from this line
250 had significantly shorter latencies to traverse the beam compared with Ahr^{b} mice. Both PCB-treated Ahr^{b}
251 groups showed impairments in this test, with more slips and more slips/step. This indicates PCB-induced
252 impairments in nigrostriatal function only in the high-affinity Ahr^{b} mice.

253 Efforts to identify a mode of action responsible for the distinct patterns of PCB-induced motor
254 impairments observed in these experiments will need to consider both the genetic differences and the
255 well-established model of noncoplanar PCB neurotoxicity mediated by the ryanodine receptor (RyR) and
256 calcium dysregulation (Dingemans et al. 2016, Pessah et al. 2010, Roegge et al. 2006, Gafni et al. 2004).
257 We note that our PCB mixture does contain noncoplanar PCBs, but the congeners included do not have
258 the same high potency at the ryanodine receptor as its prototypical agonist PCB 95. Therefore, it is likely
259 that an alternate mechanism or mechanisms are needed to explain all of the observed effects. In support of
260 that concept, Roegge et al. (2006) found increased levels of RyR1 in PCB-treated Long-Evans rats, but no

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261 evidence of increased receptor binding when looking at changes in the cerebellum. Meanwhile, Nguon et
262 al (2005) reported increased GFAP expression and reduced cerebellar mass in Sprague-Dawley rats
263 exposed to Aroclor during development. Males had more severe motor impairments on rotarod and
264 greater increases in GFAP compared with females. In contrast, we found greater impairments in female
265 mice. Piedrafita et al. (2008) identified the glutamate–NO–cGMP pathway as a cerebellar target of both
266 coplanar PCB 126 and noncoplanar PCB 153 in Wistar rats. A follow-up study confirmed motor deficits
267 for both PCB126 and PCB 153 with younger males at higher risk (Cauli et al. 2013).

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

275 Further work is needed to assess gene expression in the substantia nigra, striatum and cerebellum at
276 multiple time points to verify that there were no adverse effects on dopaminergic neurons involved in
277 motor function and to identify potential modes of action for the observed motor deficits. A histological
278 examination of those tissues could also reveal morphological changes related to abnormal development. It
279 would also be important to look at changes related to thyroid hormone depletion, because we previously
280 found that circulating thyroxine (T4) levels were reduced 80% in *Ahr*^{b}*Cyp1a2(-/-)* knockout mice
281 compared with control levels at P6 (Curran et al. 2011a). Hydroxylated PCBs can cross the placenta
282 (Meerts et al. 2002) and mimic the action of thyroid hormone (Giera et al. 2011, Darras et al. 2008).
283 Recent work has implicated CYP1A1 in metabolism of non-coplanar PCB congeners (Wadzinski et al.
284 2014, Gauger et al. 2007) to thyromimetic forms. Both 4-OH-PCB 107 (Berghuis et al. 2013) and OH-
285 PCB 106

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286 (Haijima et al. 2017) cause motor deficits, so it will be important to look into differential metabolism of
287 parent congeners in the susceptible and resistant lines of mice.

288 4.1 Conclusions.

289 Our data provides some evidence that developmental exposure to PCBs is a risk factor for Parkinson's
290 disease, but clearly demonstrate adverse effects on cerebellar development and function as well as
291 disruption of nigrostriatal pathways. The data presented here and our previous studies (Curran et al. 2011a
292 and 2011b) provide strong evidence that both *Ahr* and *Cyp1a2* genotypes affect developmental
293 neurotoxicity to polychlorinated biphenyls. We have extended our previous studies on learning and
294 memory deficits to demonstrate that high-affinity Ahr^{b}*Cyp1a2(-/-)* knockout mice are uniquely
295 susceptible to motor dysfunction following PCB exposure during gestation and lactation. By including
296 corn oil-treated control mice of three genotypes in our studies, we also uncovered a previously unreported
297 motor deficit in *Cyp1a2(-/-)* mice, regardless of *Ahr* genotype. Given known human variation in the aryl
298 hydrocarbon receptor and CYP1A2, these studies support the use of our mouse model to explore
299 developmental neurotoxicity of similar persistent organic pollutants and the role of CYP1A2 in normal
300 brain development and function.

301 5. Conflicts of interest.

302 The authors report no conflicts of interest.

303 6. Acknowledgements.

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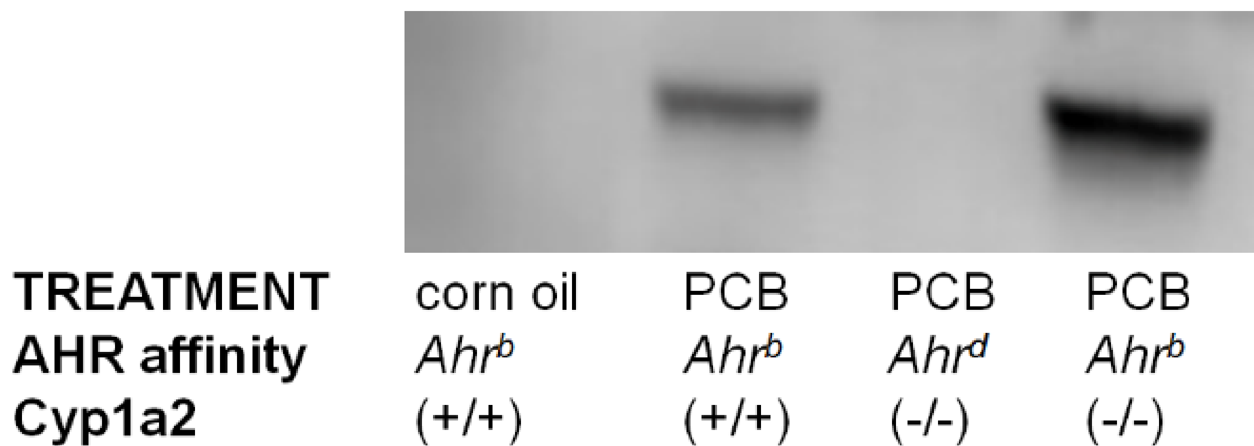

Fig. 1 Western blot of CYP1A1 in liver. CYP1A1 is an inducible enzyme typically not detectable in tissues unless the aryl hydrocarbon (AHR) is activated. Our Western blot analysis confirmed the AHR was only activated in high-affinity *Ahrb* mice compared with corn oil-treated controls and poor-affinity Ahr^d mice.

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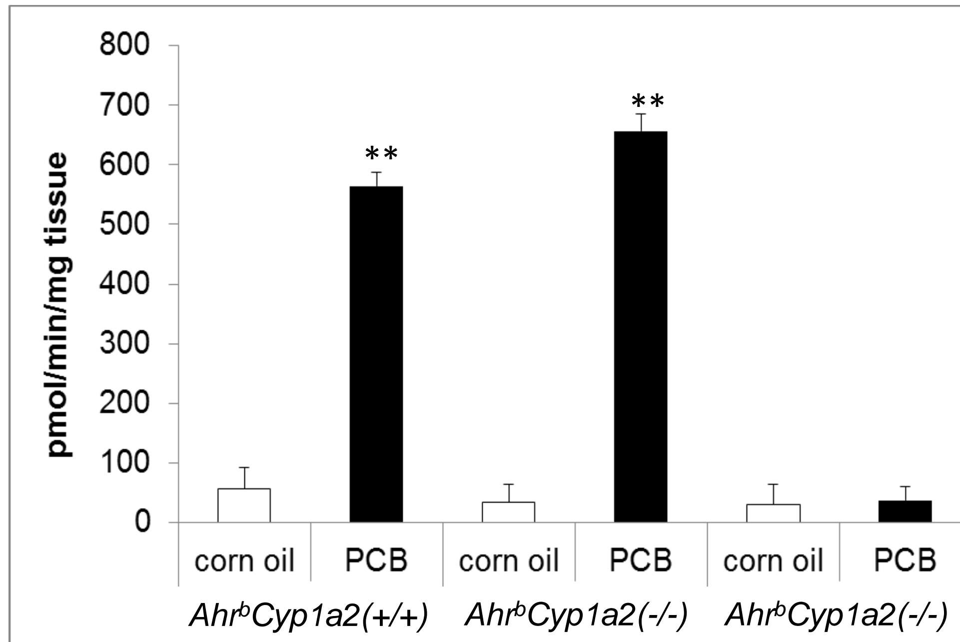

Fig. 2A EROD activity in liver. There was a significant gene x treatment interaction with higher EROD activity in liver of *Ahr*^{b} mice compared with poor-affinity Ahrd mice. N ≥ 9 per group. ** P < 0.001

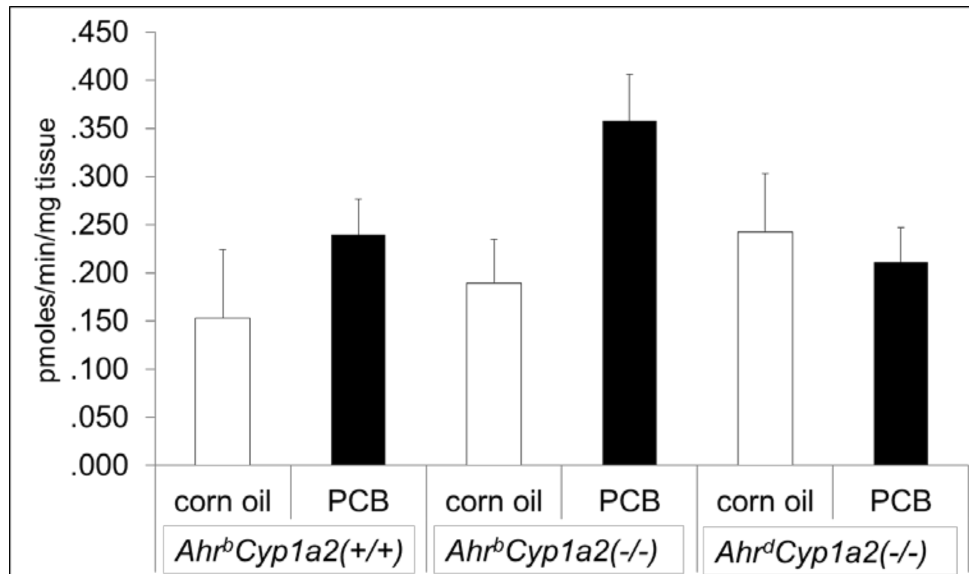

Fig 2B EROD activity in cerebellum. There was a trend for higher EROD activity in the cerebellum of AhrbCyp1a2(-/-) mice. P = 0.08. N ≥ 5 per group.

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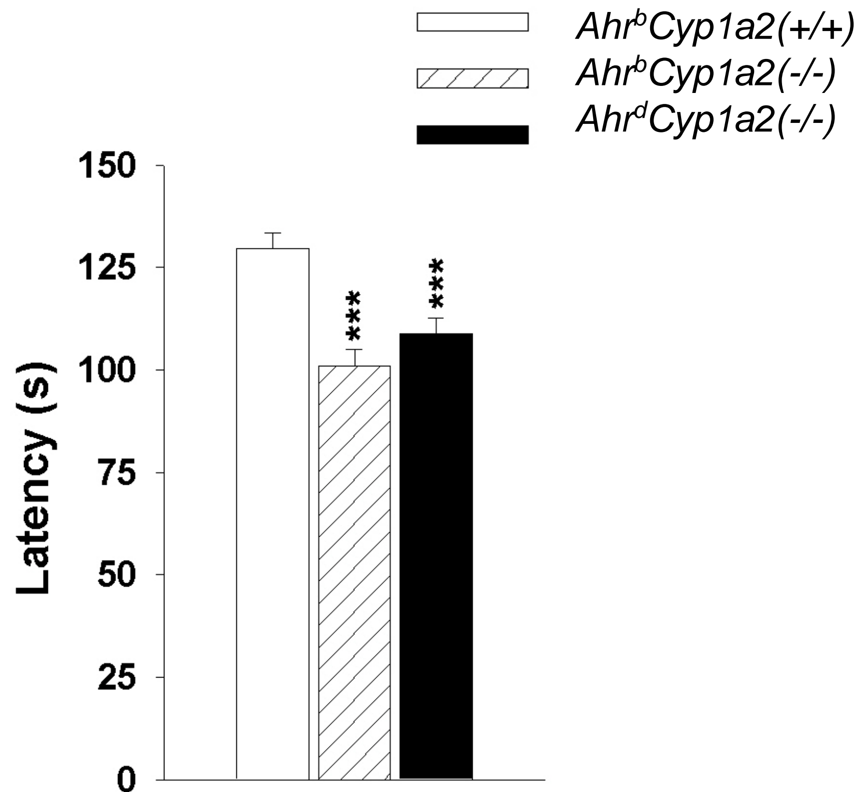

Fig. 3A Rotarod performance by genotype. There was a main effect of genotype with *Cyp1a2(-/-)* having significantly shorter latencies to fall off the rotarod. *** P < 0.001.

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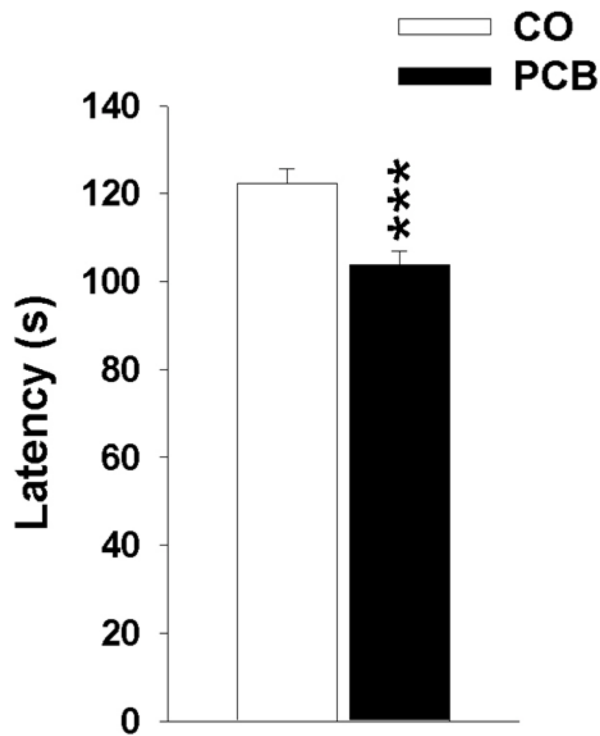

Fig. 3B Rotarod performance by treatment. There was a main effect of treatment on rotarod performance with PCB-treated mice having significantly shorter latencies to fall off the rotarod. *** $P < 0.001$.

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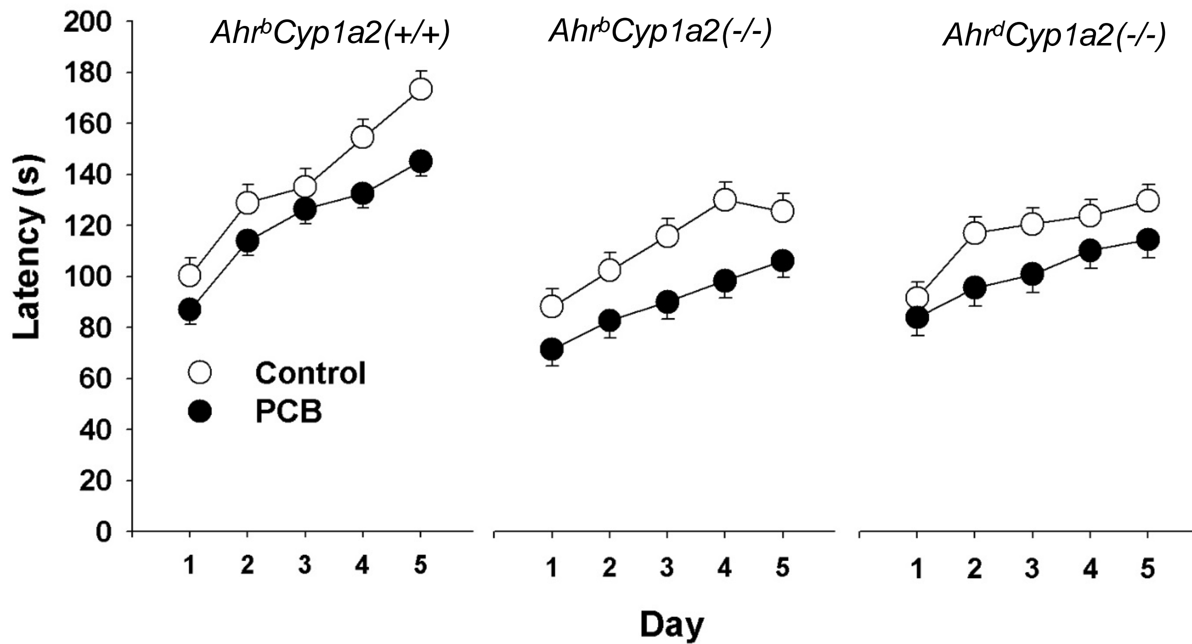

Fig. 3C Rotarod performance over time (motor learning). All groups of mice showed improvement over 5 days of testing, but high-affinity *Ahr^b^Cyp1a2(+/+)* showed the greatest motor learning compared with *Cyp1a2(-/-)* knockout mice. All PCB-treated mice showed impairments compared with their corn oil-treated controls.

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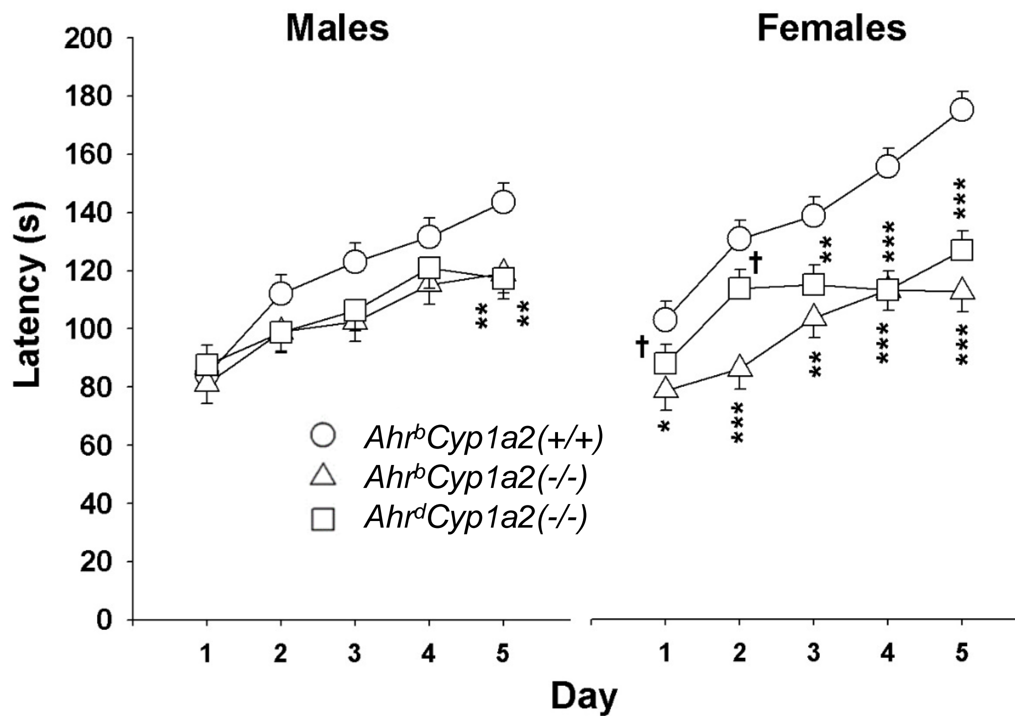

Fig. 3D. Sex differences in rotarod performance. Female mice with the *Cyp1a2(-/-)* genotype showed the greatest impairments on the rotarod test compared with *Cyp1a2(+/+)* wild type mice. *P < 0.05, ** P < 0.01, *** P <0.001.

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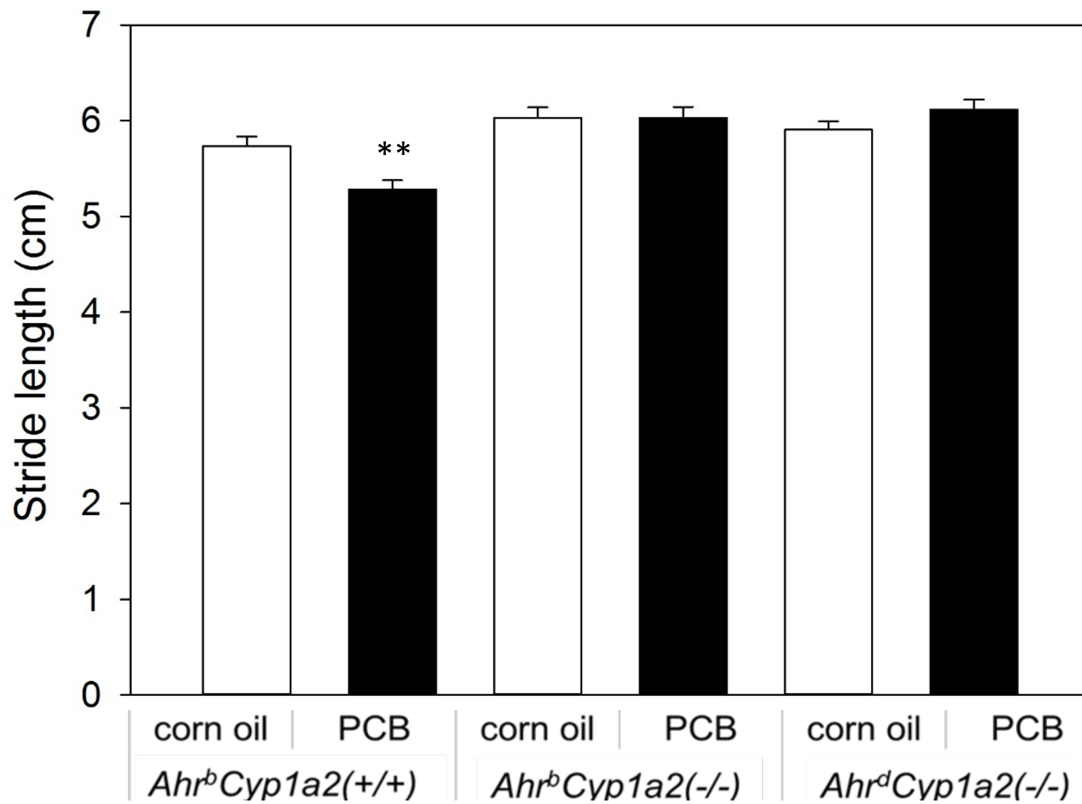

Fig. 4A. Gait stride length. PCB-treated *Ahr*^{b}*Cyp1a2(+/+)* wild type mice had significantly shorter strides than all other groups. * P < 0.01.

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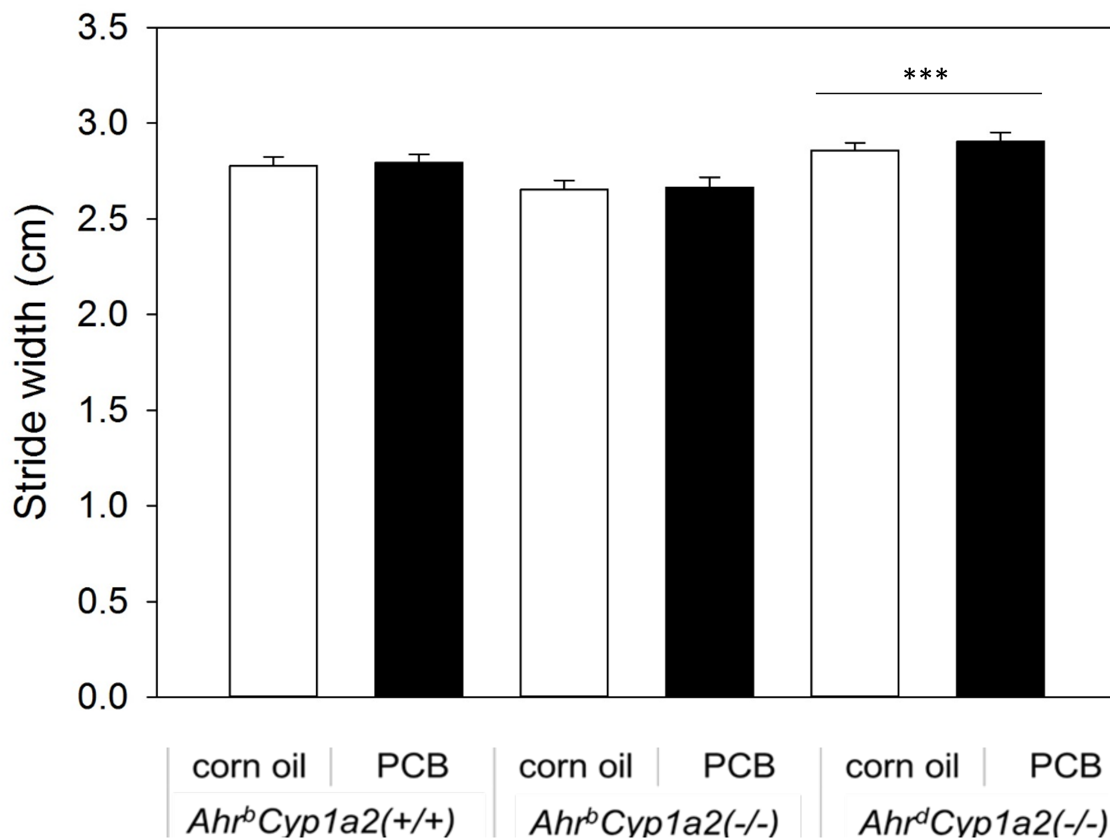

Fig. 4B. Gait stride width. *Ahr*^{d}*Cyp1a2(-/-)* mice had significantly wider strides compared with the high-affinity *Ahr*^{b} mice, but there was no effect of PCB treatment. *** $P < 0.001$

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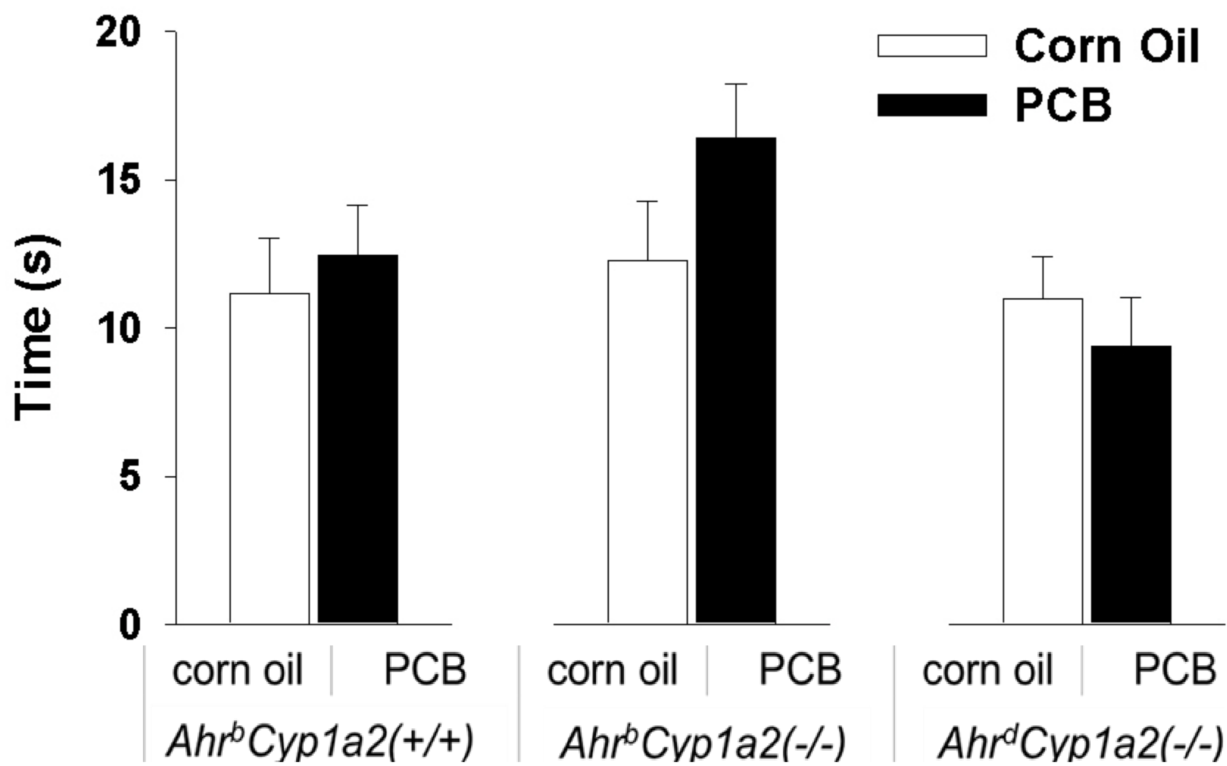

Fig. 5. Latency to remove adhesive sticker. There were no significant differences in the time required to remove a circular sticker, although PCB-treated *Ahr*^{b}*Cyp1a2(-/-)* mice had the longest latencies of all groups.

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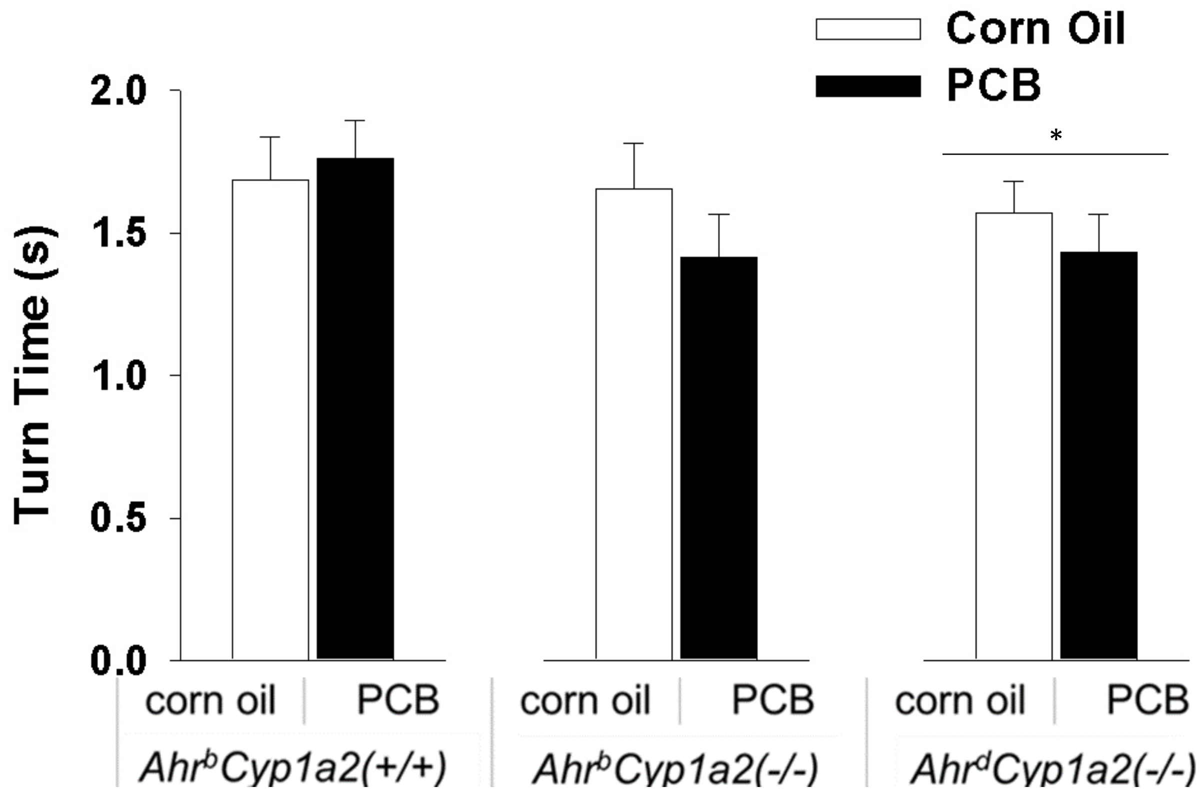

Fig. 6A Pole turn time. There was a main effect of genotype, but no effect of PCB treatment with poor-affinity *Ahr*^{d}*Cyp1a2(-/-)* mice having the shortest latencies to turn downward on the pole. * $P < 0.05$.

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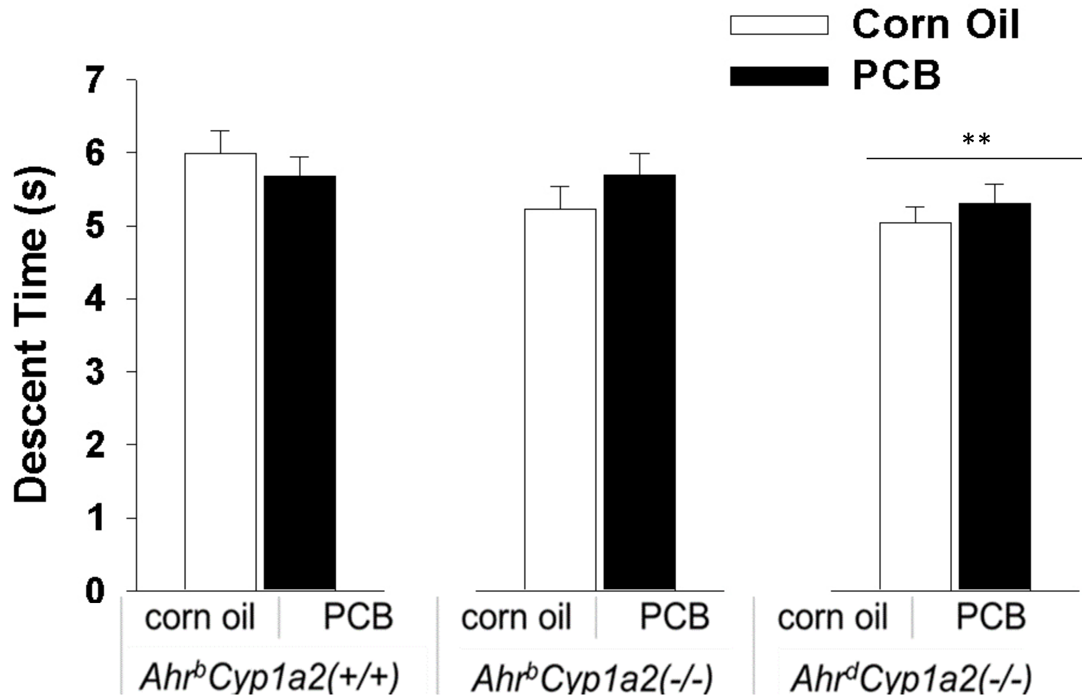

Fig. 6BA Pole descent time. There was a main effect of genotype, but no effect of PCB treatment with poor-affinity *Ahr^d Cyp1a2(-/-)* having the shortest latencies to descend the pole back to the home cage. ** $P < 0.01$.

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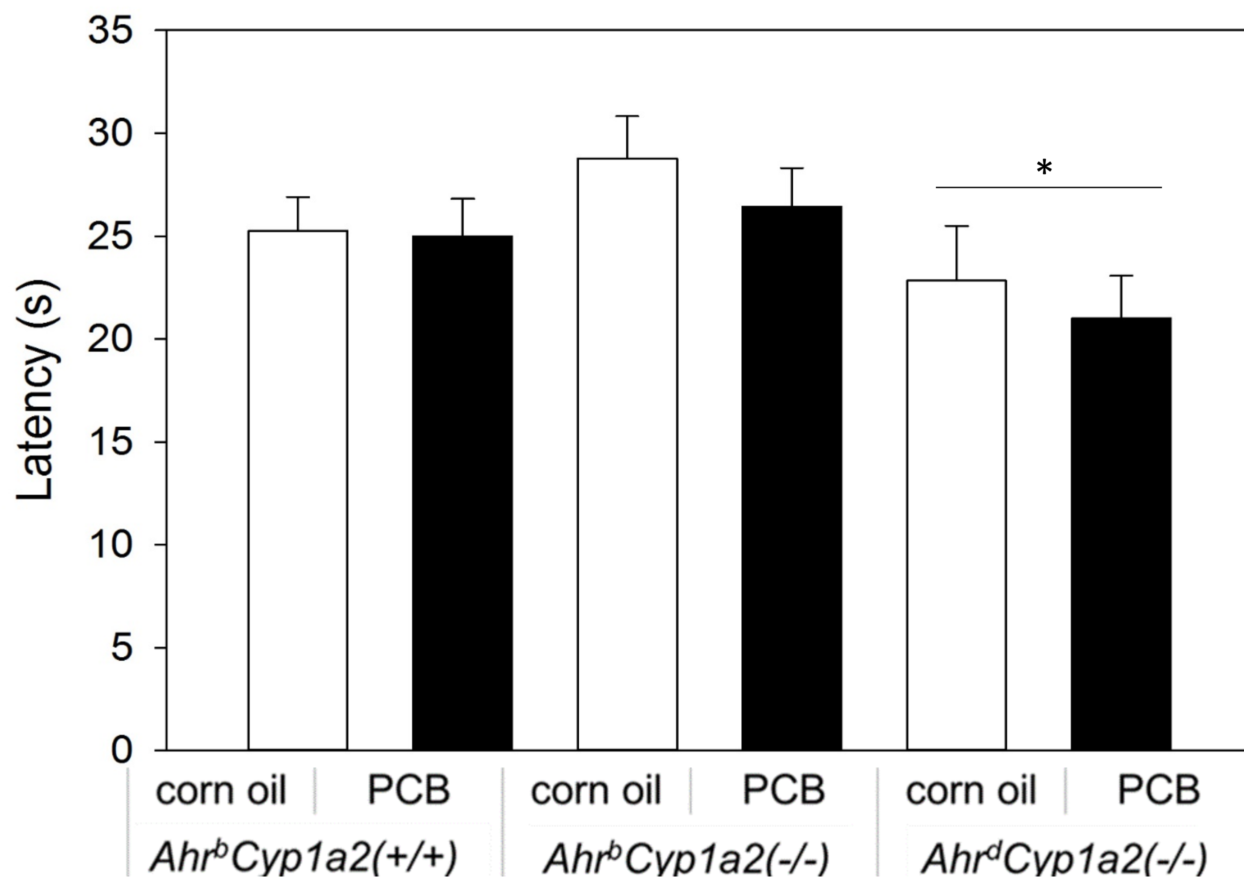

Fig. 7A. Challenging beam latency. *Ahr^d^Cyp1a2(-/-)* mice had the shortest latency to cross the balance beam, regardless of treatment. * $P < 0.05$

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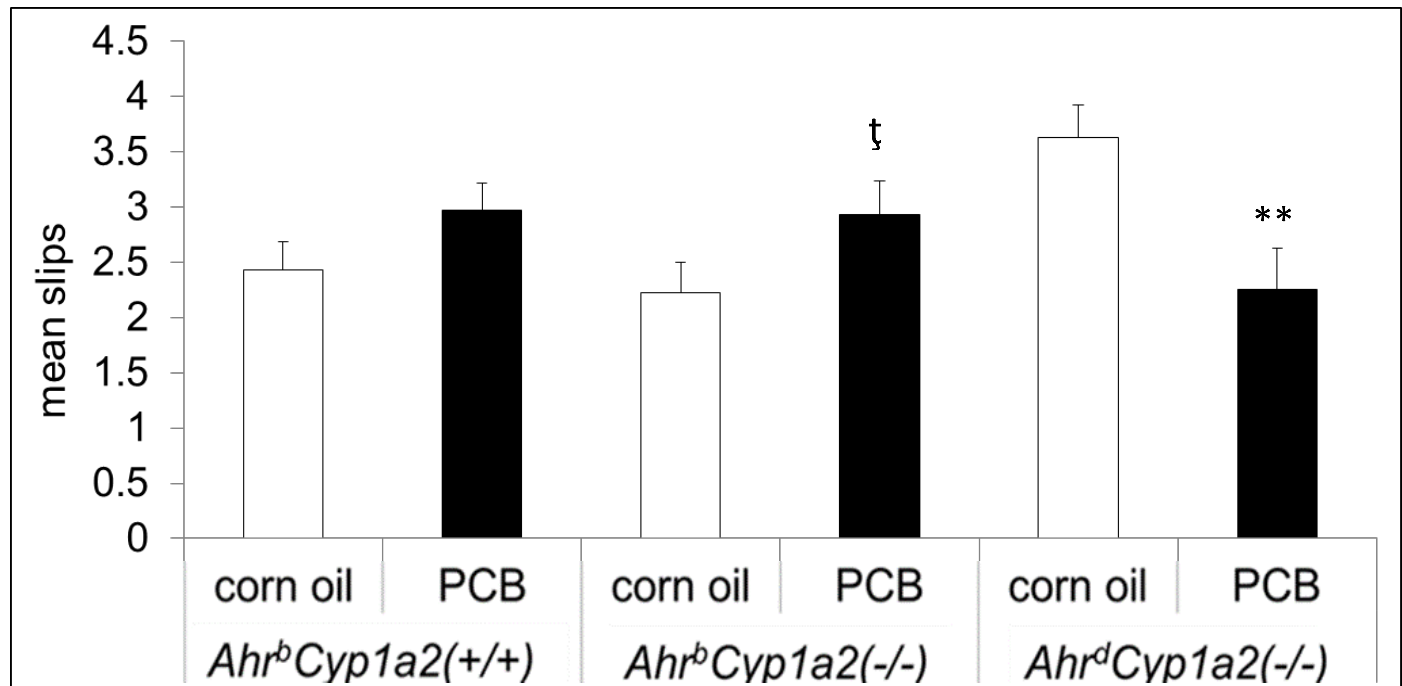

Fig. 7B Mean slips on challenging beam. There was a significant gene x treatment interaction with PCB-treated *Ahr^b* mice having more slips than corn oil-treated controls of the same genotype and poor-affinity *Ahr^d* mice having significantly fewer. ţ $P < 0.1$, ** $P < 0.01$.

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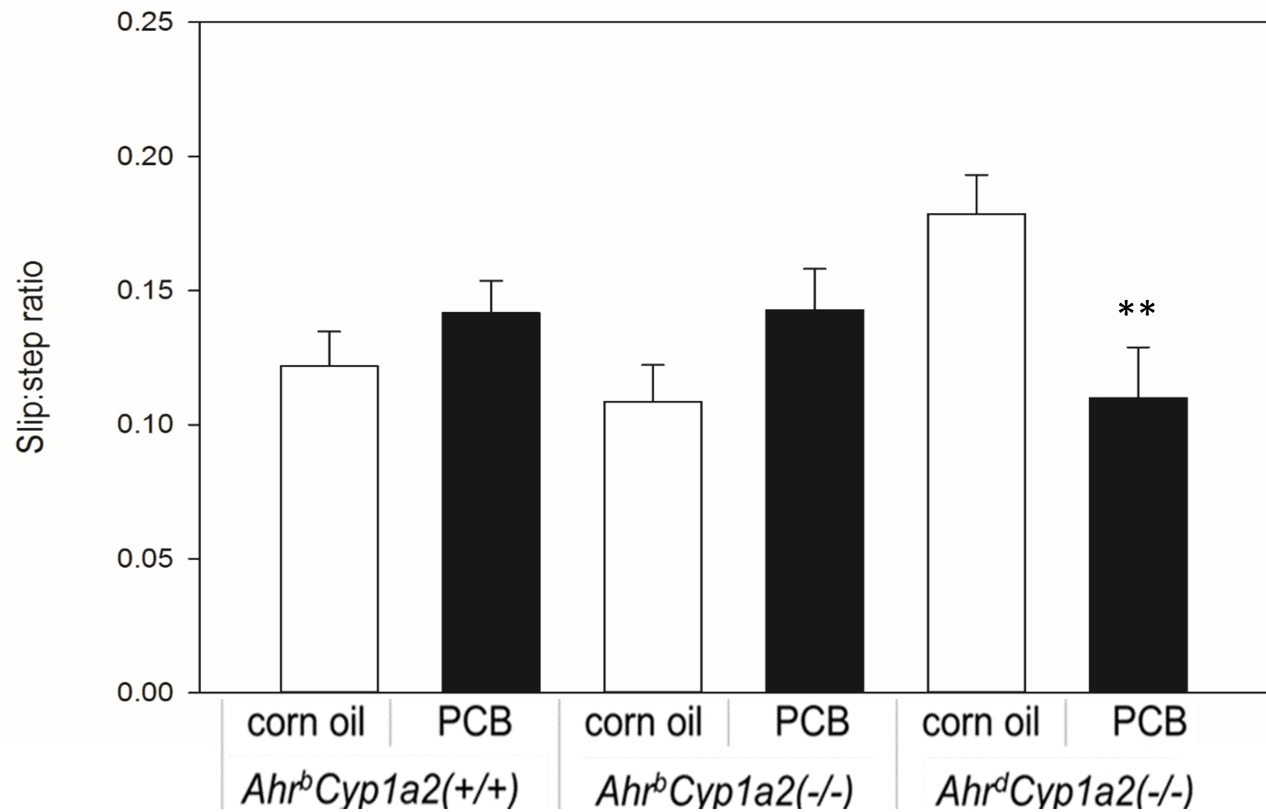

Fig. 7C Slip to Step ratio on challenging beam. There was a significant gene x treatment interaction with PCB-treated Ahr^b mice having more slips and poor-affinity Ahr^d mice having fewer. ** $P < 0.01$.