ASSESSING THE EFFECTS OF ADOLESCENT PCB EXPOSURE ON EXECUTIVE
FUNCTIONS IN HUMANS AND ANIMALS

BY

SUPIDA MONAIKUL

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Neuroscience
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2015

Urbana, Illinois

Doctoral Committee:

      Professor Susan Schantz, Chair
      Associate Professor Josh Gulley
      Professor Janice Juraska
      Associate Professor Lori Raetzman

Abstract

Converging evidence from studies in animal models and humans suggests that early developmental exposure to polychlorinated biphenyls (PCBs), a class of persistent organic pollutants, leads to deficits in cognitive functions such as cognitive flexibility and inhibitory control. These cognitive processes are mediated to a large extent by the prefrontal cortex (PFC). The dopamine (DA) system is a neurochemical system thought to play a critical role in modulating these cognitive functions, and the cognitive deficits seen with early PCB exposure may be mediated by DA dysfunction. Previous PCB studies have focused on the perinatal period as a critical period of exposure, and little or no research has examined the effects of exposure during another critical period of brain development – adolescence. During this period, the PFC and DA innervation of the PFC are undergoing marked maturation. Thus, executive functions mediated by the PFC, including cognitive flexibility and response inhibition, may be especially sensitive to disruption during adolescence. The goal of this research was to investigate the effects of adolescent PCB exposure on cognitive flexibility and response inhibition using parallel human and animal studies.

In the animal study, the goal was to examine the long-term effects of adolescent PCB exposure on cognitive flexibility, using an operant set-shifting task, and response inhibition, using a differential reinforcement of low rates of responding (DRL) task. An additional goal was to determine whether exposure during this period would lead to long-lasting changes in dopamine transporter (DAT) expression that could underlie changes in cognitive performance that were observed. One male and one female pup from each of 14 litters were assigned to each of three treatment groups, 0, 3 or 6 mg/kg/day PCBs. Rats were orally dosed daily from postnatal day (PND) 27-50 to capture the whole period of adolescence in rats, and cognitive testing began at PND 90. In the set-shifting task, we saw a sex-specific effect of PCBs on the reversal phase

with PCB-exposed males performing better in this phase, possibly due to these rats employing a simpler, more "habit-based" response strategy rather than learning the actual response reversal relevant to the task. In the DRL task, we found no effect of PCB-exposure. The PCB-related effects in performance on these two cognitive tasks were not as robust as hypothesized, and, perhaps not-surprisingly then, there were no significant differences in DAT expression in the orbital frontal cortex - which is important for reversal learning - or the striatum.

To explore effects of PCB exposure in human adolescents, data were collected from 115 12-18 year old children of sport anglers in Green Bay, Wisconsin, where this population is exposed to PCBs through consumption of fish from contaminated waters. PCB concentrations were measured in the childrens' serum and assessed in relation to performance on computerized tasks of cognitive flexibility, using the CANTAB Intradimensional/Extradimensional (ID/ED) set-shifting task, and response inhibition, using the Integrated Visual and Auditory (IVA) Continuous Performance Task. Behaviors associated with ADHD were assessed using the Conner-Wells' Behavior Rating Scale Parent Report. PCB exposure was not associated with scores on the Conners' scales, but higher PCB exposure was associated with more total trials to complete the ID/ED test in males. Higher PCB exposure, however, was not associated with a difference in performance on the response inhibition measure in boys or in girls. In summary, the results of these studies suggest there are subtle sex-specific disruptions in cognitive flexibility associated with PCB exposure during adolescence in both male rats and humans.

Acknowledgements

This project would not have been possible without the support of many people. First and foremost, I would like to give many thanks to my advisor, Susan Schantz, who has not only read my innumerable revisions but has also helped build the foundation for my scientific career. I would also like to thank my committee members, Josh Gulley, Janice Juraska and Lori Raetzman, who have offered much guidance and support throughout my graduate work. Last, but certainly not least, I would like to thank my family, especially my parents and brother, as well as numerous friends who have endured this long, long process with me, always offering me an unwavering source of support and love.

Table of Contents

Chapter 1: Background and Significance

Polychlorinated Biphenyls (PCBs)

One recurring problem in our world is the issue of enduring human health consequences of chemical use in industry and consumer products. Polychlorinated biphenyls (PCBs) are one such chemical once used as a dielectric fluid in capacitors and transformers as well as in carbonless copy paper, fluorescent light ballasts and caulking material (Ross, 2004). PCBs were banned from production in the 1970s and yet are still persistent in our environment over 4 decades later. Their lipophilicity, chemical stability and relatively long half-lives allow PCBs to biomagnify, and humans are exposed largely through consumption of contaminated fish and seafood (Crinnion, 2011). PCBs are also unintentionally produced as byproducts during pigment manufacture and are present in commercially available paint and dyed paper and plastics (Grossman, 2013). Furthermore, PCBs may be continuously re-introduced into the environment as edifices contaminated with PCB-containing building materials, such as caulking and fluorescent light ballasts, are being demolished (Hornbuckle & Robertson, 2010). It is not surprising then that despite the fact that their industrial use was discontinued in the 1970s, PCBs are still detectable in human serum (Sjodin *et al,* 2014). Furthermore, human populations that consume fish from contaminated waters such as the Great Lakes continue to show elevated PCB concentrations in blood relative to other populations (McGraw & Waller, 2009; Turyk *et al,* 2006; reviewed in Turyk *et al,* 2012).

The toxicity of PCBs became very well known when populations of individuals in Japan and Taiwan became overtly ill from consumption of rice oil that was contaminated with high levels of PCBs and polychlorinated dibenzofurans (Ross, 2004; Crinnion, 2011). However,

because these high level chemical exposures rarely occur, public health concern has shifted to the effect of low-level dietary exposures to this ubiquitous environmental contaminant.

The PCB molecule consists of two phenyl rings around which 1-10 chlorines can be substituted. Depending on the number and position of the chlorines around the phenyl rings, there can be 209 different PCB congeners with varying toxicities (Crinnion, 2011). PCB congeners can take on coplanar or non-coplanar configurations. Coplanar PCBs have two chlorines in the para-position, two or more chlorines in the meta-position and no chlorines in the ortho-position. Coplanar PCBs can activate the aryl-hydrocarbon receptor thereby exerting dioxin-like effects. Non-coplanar PCBs have one or more chlorines in the ortho-position, thus cannot assume a planar configuration. Because of this, non-coplanar PCBs have little or no affinity for the aryl hydrocarbon receptor. Non-coplanar PCBs can bind to the ryanodine receptor, a class of intracellular calcium channels found in muscle and brain tissue (Crinnion, 2011).

Individual PCB congeners are not found alone in the environment. In industrial use, PCBs were produced as commercial mixtures, known as Aroclors. These commercial formulations also do not represent the composition of environmental exposure sources due to the fact that many Aroclors were released into the environment concurrently, and that some PCB congeners break down more quickly in the environment than do others. Thus, although most studies focus on individual PCB congeners or specific Aroclor mixtures, it is most relevant to human health to study PCB toxicity in relation to actual environmental mixtures to which humans are exposed.

PCBs have been associated with adverse immune, endocrine, neurologic and reproductive effects in humans and laboratory animals (Ross, 2004; Crinnion, 2011). PCBs can affect the

developing infant through placental or lactational transfer, and pregnant women may have a pre-existing body burden as well as current exposure through fish consumption (Jacobson *et al,* 1984). Not surprisingly, there is now a wealth of literature suggesting that the perinatal period is a critical period for PCB exposure and documenting adverse effects of early exposure to PCBs, including effects on birth weight (Ross *et al,* 2004) and cognition (Eubig *et al*, 2010). In particular, early PCB exposure has been associated with deficits in executive functions including working memory, cognitive flexibility and response inhibition (reviewed by Eubig et al., 2010; Sable et al., 2006), and these executive functions are aspects of cognition thought to be mediated largely by the prefrontal cortex (reviewed in Dalley *et al,* 2004).

The Role of the PFC and DA in Cognition

The hypothesis for our proposed studies relies on the body of literature suggesting a critical role of prefrontal cortex (PFC) dopamine (DA) in modulating cognitive functions documented to be affected by perinatal PCB exposure. A large number of studies have identified an important role for the PFC in mediating executive functions, including response inhibition and cognitive flexibility. For instance, electrolytic lesion to the PFC in rats leads to impaired performance on a Differential Reinforcement of Low Rates of Responding (DRL) task, an operant task of response inhibition (Neill, 1976). Studies have also shown that lesions to the PFC impair performance on 2 other tasks of response inhibition: the stop-signal task and the go/no-go task (reviewed in Eagle & Baunez, 2010). Although the orbital frontal cortex (OFC) has been implicated in mediating performance on these tasks as well, results have been inconsistent across studies (Eagle & Baunez, 2010). Along with its role in inhibitory control, the PFC is also involved in cognitive flexibility. Lesions to the dorsolateral PFC in humans and monkeys or the medial PFC in rats have been reported to lead to deficits on set-shifting (a task engaging

cognitive flexibility) (reviewed in Floresco & Magyar, 2006). Similarly, reversible inactivation of the medial PFC resulted in robust perseverative errors when rats were required to shift strategies (Ragozzino *et al,* 1999; Floresco *et al,* 2008). Reversal learning, another aspect of cognitive flexibility, is impaired when the OFC is lesioned in rats and monkeys (McAlonan & Brown, 2003; Dias *et al,* 1996; Ghods-Sharifi *et al*, 2008). The roles different subregions of the PFC play in cognition seem to be dissociable to some extent (reviewed in Dalley *et al*, 2004). Whereas lesions to the medial PFC lead to deficits in set-shifting, medial PFC lesions do not result in deficits in reversal learning (Floresco *et al,* 2008). Likewise, lesions to the OFC result in deficits in reversal learning but not set-shifting (McAlonan & Brown, 2003).

As previously mentioned, research has suggested DA to be integral in mediating these cognitive functions. 6-hydroxydopamine (6-OHDA) lesions have been used extensively to study the impact of DA depletion in specific areas of the brain that receive DA input. For instance, one study found that 6-OHDA lesions to the PFC disrupt the ability of marmosets to perform a set-shifting task (Crofts *et al,* 2001). Furthermore, increasing extracellular DA by inhibiting catechol-O-methyltransferase (COMT) in the medial PFC of rats enhanced performance on a set-shifting task (Tunbridge *et al*, 2004). Another study assessed the effects of 6-OHDA lesions of the medial PFC on a DRL task (Sokolowski & Salamone, 1994). They found that lesioned rats responded more and received fewer reinforcers than controls, suggesting impaired response inhibition. Interestingly, humans with schizophrenia, childhood-onset psychosis or ADHD also show deficits in set-shifting (Hosenbocus & Chahal, 2012; Gualtieri & Johnson, 2008) and response inhibition (Ridderinkhof *et al,* 2004; Brodsky *et al,* 2014) among other cognitive impairments, and these deficits are thought to be due to DA system dysfunction in the frontal lobe. Furthermore, the study by Gualtieri & Johnson (2008) reported that pharmacological

treatments that increase mesocortical DA transmission (e.g., amphetamine and methylphenidate) were associated with improved performance on a set-shifting task in children with ADHD. These studies taken together indicate a critical role of mesocortical dopamine in modulating cognitive flexibility and response inhibition. Thus, exposure to environmental insults—particularly those that target the DA system—during critical periods of prefrontal cortical maturation could lead to impairments in these critical aspects of cognitive functioning.

Critical Periods of Brain Development

The complexity of brain development stems from dynamic interplays that are occurring between the developing brain and genetic and environmental influences. As such, there are critical windows of brain development and maturation during which an individual may be more sensitive to environmental exposures or conditions due to the plasticity of the nervous system, and these environmental contributions may support or disrupt development in a variety of ways. The prenatal and early postnatal period is a critical period during which rapid neurodevelopment is occurring. There is an extensive body of evidence suggesting that PCB exposure during this period can lead to long-lasting alterations in immune and endocrine system function as well as in neurodevelopmental and neurobehavioral trajectories (Crinnion, 2011).

Adolescence is another critical developmental window during which the immature brain begins to adopt its more mature, adult form and function, but there is relatively little research on the impact of environmental exposures during this period. Adolescence is characterized by robust brain plasticity and frontal lobe maturation. During this period, the brain is gradually pruning synapses and strengthening those that are maintained, cortical and limbic structures are undergoing marked remodeling, and many neurochemical systems are beginning to reach maturation (reviewed in Lenroot *et al,* 2007; Marco *et al,* 2011; Selemon, 2013.). It is believed

that many hallmarks of adolescent behavior, including impulsive and risky behavior, increased exploration and novelty-seeking, can be attributed to the developmental changes occurring during this period. Although these transient changes may be adaptive in the sense that the brain is plastic to the environment in which the individual must thrive, the increased plasticity during this time could also be detrimental in that adverse environmental conditions may be more likely to disrupt brain function during this critical period of maturation.

The prefrontal cortex during adolescence

In humans, rodents and non-human primates, the PFC is prominently remodeled during adolescence. MRI studies in humans have found that the PFC undergoes late structural changes extending into adolescence (Giedd, 2004; Sowell *et al,* 1999). Similarly, studies have found that the volume of the PFC actually decreases during adolescence in humans and rats (reviewed in Tau & Peterson, 2010). This may be due in part to robust synapse elimination that is thought to be occurring during this period. In human PFC, synaptic related proteins peak in childhood and decline during adolescence suggesting a reduction in synaptic density occurring during this period (Glantz *et al*, 2007; reviewed in Blakemore & Choudhury, 2006); however, this has been challenged recently by a study that found increasing synaptic related proteins in the PFC during adolescence (Webster *et al,* 2011). Animal studies are consistent with the human studies that observed a decline in synaptic density, with synaptic density peaking around PND31 in the PFC and declining thereafter until young adulthood (PND 60) (Gourley *et al,* 2012). In addition, one study has found that in rats, males and females show a peak in dendritic spine density in the medial PFC in early adolescence, but a reduction in spine density to that of adult levels was only seen in females (Markham *et al,* 2013). This is not surprising as sex-differences in the maturing adolescent brain have previously been reported (reviewed in Lenroot *et al,* 2010 and Juraska *et*

al, 2013). Another study looking at dendritic spine density in the medial PFC reported a similar pattern of results, but the decline in spine density in this region from adolescence to adulthood was seen in both males and females (Koss *et al,* 2014). Given that there are sex differences in the adolescent brain, it is important to continue to investigate changes in brain physiology in both males and females in order to reconcile these conflicting findings. In addition to the changes in synaptic density in the PFC, dopaminergic input to this region is also changing during adolescence. In non-human primates, DA concentrations gradually shift from the posterior to the anterior portion of the brain from the juvenile period to adolescence with concentrations highest in the PFC during adolescence (Wahlstrom *et al,* 2010). Given the increase in DA concentration in the PFC, it is not surprising that DA afferents from the ventral tegmental area that project to the PFC also increase to adult levels during adolescence (Kalsbeek, 1988; Yetnikoff *et al*, 2014). Furthermore, DA receptor densities are also changing throughout adolescence. Previous studies have reported in both rodent and nonhuman primate models that DA receptor 1 and 2 density shared similar developmental trajectories, peaking during the early postnatal period and gradually declining to adult levels during adolescence (Andersen *et al,* 2002; Weickert *et al,* 2007; Rothmund *et al,* 2012; reviewed in Wahlstrom *et al,* 2010). However, more research investigating age-related changes in DA receptor density across brain regions over the juvenile and adolescent periods is needed as studies have reported inconsistent results. Despite some remaining ambiguities, these studies suggest that the changes occurring during adolescence are dynamic and multifaceted, reflective of a system fine-tuning itself to reach its mature form.

       While dopaminergic input to the PFC is maturing during adolescence, there is concurrent maturation of cognitive functions mediated by dopaminergic input to the prefrontal cortex. In fact, studies done in humans have shown age-related improvements in executive functions

mediated by the PFC during adolescence. For instance, performance on tasks engaging executive functions such as cognitive flexibility and inhibitory control showed improvement from age 6 (childhood) to age 14 (early adolescence) in a Brazilian cohort (Dias *et al*, 2013). Research has suggested that an integration of brain function during adolescence underlies this maturation of executive functions, that is, continued myelination during this period contributes to the ability of the PFC to influence the rest of the brain (Olesen *et al,* 2003; reviewed in Luna 2009). One method that has been used to investigate functional circuits involved in cognitive processing across the lifespan is neuroimaging. fMRI studies have found that performance on tasks of response inhibition that engage the PFC is poor in childhood but steadily improves with age, reaching adult levels of performance in mid to late adolescence (Luna *et al,* 2010). Another study examined functional connectivity during a go/no-go task of response inhibition and found changes in the fronto-striatal-thalamic network activity from adolescence (age 11-17) to adulthood (age 18-37), and this was related to improvements in performance across these age groups (Stevens *et al,* 2007). Another study using a different neuroimaging technique, diffusion tensor imaging, found that as the cingulum, a white matter tract projecting from the cingulate gyrus to the entorhinal cortex, increases in fiber density and myelination from childhood to adulthood, performance on tasks of executive function improves (Peters *et al,* 2014). Taken together, research done in humans has shown not only that executive functioning is maturing throughout adolescence but also that functional connectivity of the frontal regions to other areas of the brain may be associated with these improvements in executive functioning.

A maturation in executive control is also seen between adolescence and adulthood in animal models. Adolescent rats as compared to adult rats show poorer performance on tasks engaging the PFC, including tests of response inhibition (Andrezejewski *et al*, 2011) and

behavioral set-shifting (Newman & McGaughy, 2011). Like in humans, the volume of the rat PFC decreases during adolescence due to synaptic pruning and neuron loss (Markham *et al,* 2007) while white matter volume in the frontal lobe is increasing due to increasing myelination in the cortex (Juraska & Markham, 2004). As described above, functional connectivity of relevant regions of cortex, including the PFC, plays a critical role in executive functioning. Taken together, these human and animal studies provide strong evidence that the frontal lobe is undergoing marked remodeling and maturation during adolescence, and these changes contribute to the differences in cognitive performance seen between adolescents and adults. Exposure to a neurotoxicant such as PCBs during this critical period may lead to disruption of prefrontal cortical maturation and DA system dysfunction, which may then alter cognitive and behavioral functioning later in life.

Neurotoxicity of PCBs

Animal studies of perinatal PCB exposure and cognition

As discussed above, there are vulnerable developmental periods during which environmental influences can greatly impact brain development, behavior and cognition. Although the brain is robustly developing and maturing during both the perinatal period and the adolescent period, many studies that have assessed the neurotoxicity of PCBs have focused primarily on perinatal exposure because of the likelihood of maternal transfer of this chemical. The developing fetus may be exposed *in utero* via placental transfer, and the developing infant may be exposed postnatally to PCBs through breastmilk (Jacobson *et al,* 1984).

Because there are many confounding factors in epidemiological studies, animal models have been essential for more directly examining neurobehavioral effects of developmental insults such as exposure to chemical contaminants. Some aspects of cognition that have been tested in

animal models include executive functions such as working memory, cognitive flexibility and response inhibition. Monkeys exposed to an experimental PCB mixture during the early postnatal period showed impaired acquisition of a delayed spatial alternation (DSA) task, responding repeatedly on a lever instead of alternating responses (Rice & Hayward, 1997). This perseverative responding on the DSA task has also been seen in another study in which monkeys were perinatally exposed to a commercial PCB mixture (Levin *et al,* 1988) as well as in a rat study in which adult offspring exposed gestationally and lactationally to a commercial PCB mixture were tested (Widholm *et al,* 2004). Perseverative responding in perinatally PCB-exposed rats has also been seen on a discrimination reversal learning task that assesses how quickly and effectively a subject can alter a response strategy when a response contingency changes (Widholm *et al,* 2001).

Along with these impairments in cognitive flexibility, early PCB exposure also impairs response inhibition in rodents and monkeys. The studies further described employ the DRL task in which the subject must wait a fixed period of time before responding in order to gain a reward. Rice (1998) dosed monkeys postnatally with a PCB mixture representative of PCBs typically found in breast milk. The PCB exposed animals showed a reduced ratio of reinforced to nonreinforced responses on the DRL task as compared to controls, with PCB-exposed monkeys making a greater number of non-reinforced responses and thus earning fewer reinforcers. Similarly, adult rats perinatally exposed to an environmental PCB mixture showed a decrease in the ratio of reinforced to nonreinforced presses as compared to non-treated controls during the DRL task (Sable *et al*, 2009), although these findings were not consistent across all rodent studies looking at PCB exposure and DRL performance (e.g. Sable *et al,* 2006). Overall, these studies suggest that early PCB exposure impairs the animal's ability to withhold responding for a

constant interval of time across test sessions. In summary, a number of studies in monkeys and rats have found that early developmental exposure to commercial mixtures of PCBs results in deficits in cognitive flexibility and response inhibition that last into adulthood and continue to be apparent well after exposure has ceased.

Humans studies of perinatal PCB exposure and cognition

Because humans are directly exposed to this environmental toxicant, many studies have evaluated the association between PCB exposure and neurodevelopmental outcomes. Although the human data are less definitive due to the many confounding variables present in our complex environment; much of the research does suggest an association between PCB exposure and neurodevelopmental deficits (Boucher *et al.,* 2009; Eubig *et al*., 2010).

In a Michigan birth cohort of children born to women who consumed PCB-contaminated fish from Lake Michigan, higher umbilical cord serum PCB levels were dose-dependently associated with poorer performance on Fagan's test of visual recognition memory at 7 months of age and poorer verbal and quantitative short-term memory function at 4 years of age (Jacobson *et al,* 1985; Jacobson *et al,* 1990). Prenatal PCB exposure in this cohort was also associated with poorer concentration, greater impulsivity, poorer verbal, pictorial, and auditory working memory (Jacobson & Jacobson, 1996) as well as lower full-scale and verbal IQ scores with the strongest effects related to memory, cognitive flexibility and attention when the children were tested at 11 year of age (Jacobson & Jacobson, 2003).

An Oswego, New York birth cohort that included children born to women who consumed PCB-contaminated Lake Ontario fish found similar results. Prenatal PCB exposure, as measured in umbilical cord serum, was associated with poorer performance on the Fagan's test of infant intelligence at 6 and 12 months of age (Darvill *et al,* 2000). Stewart and colleagues also

performed a series of cognitive tests on children of this cohort across different time points. They found cord serum PCB levels to be predictors of subtle deficits in performance on the McCarthy Scales of Children's Abilities at 38 months of age (Stewart *et al*, 2003). They also found a dose-dependent association between prenatal PCB exposure and excessive (impulsive) responding on a task of response inhibition at 4.5 years of age (Stewart *et al,* 2003). When tested at 8 and 9.5 years of age, a similar association was seen between prenatal PCB exposure and poorer performance on a similar task of response inhibition (Stewart *et al,* 2005).

Although many populations exposed to PCBs have been studied, the findings may be confounded by other variables such as multiple co-occurring toxicant exposures (Korrick & Sagiv, 2008). For instance, birth cohort studies in the Faroe Islands have been conducted evaluating the association between PCB and methylmercury (MeHg) exposure and neurodevelopment. The Faroe Islands are North Atlantic Danish islands where the traditional diet includes whale meat and blubber, leading to high levels of MeHg and PCB exposure, respectively (Grandjean *et al*, 2001). This co-occuring exposure can complicate interpretation of the results. For example, 7 year old children with higher prenatal PCB levels showed poorer performance on tests of attention and verbal ability, but the associations were no longer significant after adjustment for MeHg levels. Moreover, neuroprotective nutrients in fish and marine mammals such as selenium, docosahexaenoic acid (DHA) and omega-3 polyunsaturated fatty acids can further confound results. Much like the Faroe Island cohort, Inuit families living in Northern Quebec are exposed to both PCBs and MeHg through a diet that includes marine mammals. In one study conducted in infants prenatally exposed to these contaminants, significant associations were found between exposure and poorer cognitive functioning only when controlling for seafood nutrients measured in subjects' blood samples (Boucher *et al,*

2014). All in all, although these studies suggest that PCB exposure from fish is associated with detrimental cognitive outcomes, the complexities of interpretation created by co-occuring contaminant exposures together with the potentially neuroprotective effects of nutrients in fish and marine mammals highlight the need for complementary, carefully controlled animal studies to support epidemiological evidence of PCB neurotoxicity.

Effects of adolescent PCB exposure on cognition

Based on the plasticity of the frontal lobe during adolescence, exposure to a neurotoxicant such as PCBs during this period may disrupt the maturation of neurochemical systems as well as the behaviors and cognitive functions mediated by them. Unfortunately, there is very limited research in humans or animals examining the relationship between adolescent PCB exposure and either cognition or neurochemistry. One epidemiological study assessed cognitive function in a cohort of Mohawk adolescents 10-17 years of age and found that recent environmental exposure to PCBs (e.g., through fish consumption) was associated with poorer scores on cognitive tests of delayed recall, long term retrieval and comprehension-knowledge (Newman *et al*, 2009). Although the aspects of cognitive function examined in the study were different from those addressed in our studies, the results do provide evidence that recent PCB exposure in adolescents may be associated with poorer performance on cognitive tasks.

Surprisingly, almost no animal research assessing PCB exposure beyond early puberty exists in the literature. One study looked at the effects of exposure to an individual non-coplanar PCB congener (PCB 153) at three time points between birth and puberty and reported deficits in attention and impulsivity when rats were tested from mid-adolescence to adulthood on a variable interval operant task (Johansen *et al,* 2014). However, these data were significant only in the spontaneously hypertensive rat, a rat model bred for impulsive and inattentive behaviors, and not

in a normal outbred strain (Wistar Kyoto). Thus, it is possible that PCB exposure in this instance was exacerbating pre-existing cognitive deficits. Also, this study dosed at around postnatal days 8, 14 and 20, so it does not provide any information on the effects of exposure during adolescence, per se.

Effects of PCB Exposure on Neurochemistry

Much research has focused on the neurochemical mechanisms that may underlie the cognitive and behavioral deficits seen with perinatal PCB exposure. This attention has focused primarily on the effects of PCBs on the dopamine (DA) system, and many *in vivo* and *in vitro* studies have reported reductions or alterations in brain DA function following exposure to PCBs. These studies all use commercial mixtures with mostly ortho-substituted non-coplanar PCBs or individual congeners, and thus, do not closely mimic typical human exposure.

In vitro studies have found that Aroclor mixtures as well as individual non-coplanar PCB congeners induce decreases in DA release (Angus & Contreras, 1996) and synaptosomal DA content (Bemis & Seegal, 2004) in treated cells. Another study using organotypic co-cultures of rat striatum and ventral midbrain, regions that contain populations of DA neurons, reported an overall increase in neuronal death in both regions and a reduction in DA neurons in the ventral midbrain using an environmentally relevant "Fox River" PCB mixture that has been used extensively in our studies (Lyng *et al*, 2007). This suggests that a PCB-induced loss of DA neurons may contribute to decreased DA levels seen in other studies.

Numerous *in vivo* studies have reported findings similar to those seen in *in vitro* studies. Lee and colleagues (2012) dosed adult rats for 4 weeks with a low-dose exposure to a commercial Aroclor mixture and saw an increase in oxidative stress in DA neurons and ultimately a loss of DA neurons in the substantia nigra and ventral tegmental area, regions of the

brain containing DA cell bodies, suggesting permanent DA cell loss due to PCB exposure. Reductions in DA concentrations have also been found in the striatum of rats (Seegal *et al,* 2002) and non-human primates (Seegal *et al,* 1991) following adult exposure to commercial Aroclor mixtures. DA decreases in the striatum have also been reported in weanling rats perinatally exposed to a non-coplanar PCB congener (Castoldi *et al,* 2006). More in line with deficits in executive functions seen in PCB-exposed humans and animals, one study found that rats perinatally exposed to a non-coplanar PCB congener showed a reduction in frontal cortical DA levels that persisted into adulthood (Seegal *et al,* 2005). This piece of evidence in combination with the PCB-induced reduction in the number of DA neurons in the ventral midbrain that project to the prefrontal cortex suggest that PCB exposure could lead to hypofunction of the DA system in the prefrontal cortex.

The dopamine transporter (DAT), responsible for reuptake of DA into the presynaptic cell, and the vesicular monoamine transporter (VMAT2), responsible for repackaging cytosolic DA into vesicles for later release, are both thought to be inhibited by PCB exposure (Bemis & Seegal, 2004; Lyng *et al*, 2007). These results were found with commercial Aroclor mixtures (Bemis & Seegal, 2004), environmentally relevant mixtures (Lyng *et al*, 2007), and individual non-coplanar congeners (Bemis & Seegal, 2004). Both human (Seegal *et al,* 2010) and animal (Bemis & Seegal, 2004; Caudle *et al,* 2006; Fonnum *et al,* 2006; Lyng *et al,* 2007; Richardson & Miller, 2004) studies have reported a reduction in DAT and VMAT2 expression following PCB exposure. The effects of PCBs on DAT and VMAT2 likely reflect the reduction in DA levels reported in numerous studies. Exposures to PCB mixtures and noncoplanar congeners have also been found to alter concentrations of other neurochemicals such as norepinephrine, serotonin, glutamate, and GABA, but the effects on the DA system seem to be most consistently studied

and replicated (Mariussen & Fonnum, 2001; Seegal *et al,* 1985). Furthermore, it seems that the DA system is more sensitive to disruption by PCBs than the other neurotransmitter systems (Mariussen & Fonnum, 2001). In support of this, one study has shown that DA cells are more susceptible to oxidative injury due to exposure to a commercial PCB mixture than are non-dopaminergic cells (Lee & Opanashuk, 2004). All in all, the evidence presented suggests that noncoplanar congeners and PCB mixtures induce DA dysfunction that may be an underlying mechanism contributing to the cognitive deficits reported with PCB-exposure.

Conclusions

Although there is a wealth of human and animal research studying the impact of perinatal PCB exposure on brain and behavior, there is very little research examining the impact of exposure during another critical period of brain development – adolescence. As reviewed above, executive functions, the PFC, and DA input to the PFC are maturing during this period. Perinatal PCB exposure has been shown to target both executive functions and the DA system. Thus, PCB exposure during adolescence may adversely affect mesocortical DA system development, resulting in deficits in executive functions mediated by the PFC. The findings from the studies outlined below will provide important evidence regarding the effects of adolescent PCB exposure on brain neurochemistry and cognition. More importantly, if consistent results are obtained in these parallel human and animal studies, this will provide stronger evidence that PCB exposure during adolescence is a human health risk than would either approach alone.

References

Andersen, S.L., Thompson, A.P., Krenzel, E., & Teicher, M.H. (2002). Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. *Psychoneuroendocrinology.* 27(6):683-91.

Andrzejewski, M. E., Schochet, T. L., Feit, E. C., Harris, R., McKee, B. L., & Kelley, A. E. (2011). A comparison of adult and adolescent rat behavior in operant learning, extinction, and behavioral inhibition paradigms.*Behavioral Neuroscience, 125*(1), 93-105.

Angus, W. G., & Contreras, M. L. (1996). Effects of polychlorinated biphenyls on dopamine release from PC12 cells. *Toxicology Letters, 89*(3), 191-199.

Bemis, J. C., & Seegal, R. F. (2004). PCB-induced inhibition of the vesicular monoamine transporter predicts reductions in synaptosomal dopamine content. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 80*(2), 288-295.

Blakemore, S.J. & Choudhury, S. (2006). Development of the adolescent brain: implications for executive function and social cognition. *J Child Psychol Psychiatry*. 47(3-4):296-312. Review.

Boucher, O., Bastien, C. H., Saint-Amour, D., Dewailly, E., Ayotte, P., Jacobson, J. L., et al. (2010). Prenatal exposure to methylmercury and PCBs affects distinct stages of information processing: An event-related potential study with inuit children. *Neurotoxicology, 31*(4), 373-384.

Boucher, O., Muckle, G., & Bastien, C. H. (2009). Prenatal exposure to polychlorinated
biphenyls: A neuropsychologic analysis. *Environmental Health Perspectives, 117*(1), 7-
16.

Boucher, O., Muckle, G., Jacobson, J. L., Carter, R. C., Kaplan-Estrin, M., Ayotte, P., et al.
(2014). Domain-specific effects of prenatal exposure to PCBs, mercury, and lead on
infant cognition: Results from the environmental contaminants and child development
study in nunavik. *Environmental Health Perspectives, 122*(3), 310-316.

Brodsky, K., Willcutt, E.G., Davalos, D.B., & Ross, R.G. (2014). Neuropsychological
functioning in childhood-onset psychosis and attention-deficit/hyperactivity disorder. *J
Child Psychol Psychiatry.* 55(7):811-8.

Castoldi, A. F., Blandini, F., Randine, G., Samuele, A., Manzo, L., & Coccini, T. (2006). Brain
monoaminergic neurotransmission parameters in weanling rats after perinatal exposure to
methylmercury and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153). *Brain
Research, 1112*(1), 91-98.

Caudle, W. M., Richardson, J. R., Delea, K. C., Guillot, T. S., Wang, M., Pennell, K. D., et al.
(2006). Polychlorinated biphenyl-induced reduction of dopamine transporter expression
as a precursor to parkinson's disease-associated dopamine toxicity. *Toxicological
Sciences : An Official Journal of the Society of Toxicology, 92*(2), 490-499.

Crews, F., He, J., & Hodge, C. (2007). Adolescent cortical development: a critical period of
vulnerability for addiction. *Pharmacol Biochem Behav.* 86(2):189-99. Review.

Crinnion, W. J. (2011). Polychlorinated biphenyls: Persistent pollutants with immunological, neurological, and endocrinological consequences. *Alternative Medicine Review : A Journal of Clinical Therapeutic, 16*(1), 5-13.

Crofts, H.S., Dalley, J.W., Collins, P., Van Denderen, J.C., Everitt, B.J., Robbins, T.W., & Roberts, A.C. (2001). Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. *Cereb Cortex.* 11(11):1015-26.

Dalley, J.W., Cardinal, R.N., & Robbins, T.W. (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev.* 28(7):771-84. Review.

Darvill, T., Lonky, E., Reihman, J., Stewart, P., & Pagano, J. (2000). Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. *Neurotoxicology, 21*(6), 1029-1038.

Dias, N.M., Menezes, A., & Seabra, A.G. (2013). Age differences in executive functions within a sample of Brazilian children and adolescents. *Span J Psychol.*16:E9.

Dias, R., Robbins, T.W. & Roberts, A.C. (1996). Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behav Neurosci.* 110(5):872-86.

Eagle, D.M. & Baunez, C. (2010). Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. *Neurosci Biobehav Rev.* 34(1):50-72.

Eubig, P. A., Aguiar, A., & Schantz, S. L. (2010). Lead and PCBs as risk factors for attention Deficit/Hyperactivity disorder. *Environmental Health Perspectives, 118*(12), 1654-1667.

Floresco, S. B., Block, A. E., & Tse, M. T. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural Brain Research, 190*(1), 85-96.

Floresco, S.B. & Magyar O. (2006). Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology (Berl).* 188(4):567-85. Review.

Fonnum, F., Mariussen, E., & Reistad, T. (2006). Molecular mechanisms involved in the toxic effects of polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs). *Journal of Toxicology and Environmental Health.Part A, 69*(1-2), 21-35.

Ghods-Sharifi, S., Haluk, D.M., & Floresco, S.B. (2008). Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. *Neurobiol Learn Mem.* 89(4):567-73.

Giedd, J.N. (2004). Structural magnetic resonance imaging of the adolescent brain. *Ann N Y Acad Sci.* 1021:77-85.

Glantz, L.A., Gilmore, J.H., Hamer, R.M., Lieberman, J.A., & Jarskog, L.F. (2007).

   Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from

   mid-gestation into early adulthood. *Neuroscience*. 149(3):582-91.

Grandjean, P., Weihe, P., Burse, V. W., Needham, L. L., Storr-Hansen, E., Heinzow, B., et al.

   (2001). Neurobehavioral deficits associated with PCB in 7-year-old children prenatally

   exposed to seafood neurotoxicants. *Neurotoxicology and Teratology, 23*(4), 305-317.

Grossman, E. (2013). Nonlegacy PCBs: pigment manufacturing by-products get a second look.

   Environ Health Perspect. 121(3):A86-93.

Gualtieri, C.T. & Johnson, L.G. (2008). Medications do not necessarily normalize cognition in

   ADHD patients. *J Atten Disord.* 11(4):459-69.

Hornbuckle, K. & Robertson, L. (2010). Polychlorinated biphenyls (PCBs): sources, exposures,

   toxicities. *Environ Sci Technol*. 44(8):2749-51.

Hosenbocus, S. & Chahal R. (2012). A review of executive function deficits and

   pharmacological management in children and adolescents. *J Can Acad Child Adolesc

   Psychiatry.* 21(3):223-9.

Huttenlocher, P. R. (1984). Synapse elimination and plasticity in developing human cerebral

   cortex. *American Journal of Mental Deficiency, 88*(5), 488-496.

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex

   development. *Neuropsychologia, 28*(6), 517-527.

Jacobson, J. L., Fein, G. G., Jacobson, S. W., Schwartz, P. M., & Dowler, J. K. (1984). The

transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs)

across the human placenta and into maternal milk. *American Journal of Public*

Health, 74(4), 378-379.

Jacobson, J. L., & Jacobson, S. W. (1996). Dose-response in perinatal exposure to

polychlorinated biphenyls (PCBs): The michigan and north carolina cohort

studies. *Toxicology and Industrial Health, 12*(3-4), 435-445.

Jacobson, J. L., & Jacobson, S. W. (1996). Intellectual impairment in children exposed to

polychlorinated biphenyls in utero. *The New England Journal of Medicine, 335*(11), 783-

789.

Jacobson, J. L., & Jacobson, S. W. (2003). Prenatal exposure to polychlorinated biphenyls and

attention at school age. *The Journal of Pediatrics, 143*(6), 780-788.

Jacobson, J. L., Jacobson, S. W., & Humphrey, H. E. (1990). Effects of exposure to PCBs and

related compounds on growth and activity in children. *Neurotoxicology and*

Teratology, 12(4), 319-326.

Jacobson, S. W., Fein, G. G., Jacobson, J. L., Schwartz, P. M., & Dowler, J. K. (1985). The

effect of intrauterine PCB exposure on visual recognition memory. *Child*

Development, 56(4), 853-860.

Johansen, E. B., Fonnum, F., Lausund, P. L., Walaas, S. I., Baerland, N. E., Woien, G., et al.

(2014). Behavioral changes following PCB 153 exposure in the spontaneously

hypertensive rat - an animal model of attention-Deficit/Hyperactivity

disorder. *Behavioral and Brain Functions : BBF, 10*, 1-9081-10-1.

Juraska, J.M. & Markham, J.A. (2004). The cellular basis for volume changes in the rat cortex

during puberty: white and gray matter. *Ann N Y Acad Sci*. 1021:431-5.

Juraska, J.M., Sisk, C.L., & DonCarlos, L.L. (2013). Sexual differentiation of the adolescent

rodent brain: hormonal influences and developmental mechanisms. *Horm Behav*.

Jul;64(2):203-10.

Kalsbeek, A. (1988). Development of the dopaminergic innervation in the prefrontal cortex of

the rat. *Journal of Comparative Neurology, 269*(1), 58.

Korrick, S. A., & Sagiv, S. K. (2008). Polychlorinated biphenyls, organochlorine pesticides and

neurodevelopment. *Current Opinion in Pediatrics, 20*(2), 198-204.

Koss, W.A., Belden, C.E., Hristov, A.D., & Juraska, J.M. (2014). Dendritic remodeling in the

adolescent medial prefrontal cortex and the basolateral amygdala of male and female rats.

Synapse. 68(2):61-72.

Kostyniak, P. J., Hansen, L. G., Widholm, J. J., Fitzpatrick, R. D., Olson, J. R., Helferich, J. L.,

et al. (2005). Formulation and characterization of an experimental PCB mixture designed

to mimic human exposure from contaminated fish. *Toxicological Sciences : An Official

Journal of the Society of Toxicology, 88*(2), 400-411.

Lee, D.W. & Opanashuk, L.A. (2004). Polychlorinated biphenyl mixture aroclor 1254-induced

oxidative stress plays a role in dopaminergic cell injury. *Neurotoxicology*. 25(6):925-39.

Lee, D. W., Notter, S. A., Thiruchelvam, M., Dever, D. P., Fitzpatrick, R., Kostyniak, P. J., et al. (2012). Subchronic polychlorinated biphenyl (aroclor 1254) exposure produces oxidative damage and neuronal death of ventral midbrain dopaminergic systems. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 125*(2), 496-508.

Lenroot, R.K., Gogtay, N., Greenstein, D.K., Wells, E.M., Wallace, G.L., et al. (2007). Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage*. 36(4):1065-73.

Lenroot, R.K. & Giedd, J.N. (2010). Sex differences in the adolescent brain. Brain Cogn. 72(1):46-55.

Levin, E. D., Schantz, S. L., & Bowman, R. E. (1988). Delayed spatial alternation deficits resulting from perinatal PCB exposure in monkeys. *Archives of Toxicology, 62*(4), 267-273.

Luna B. (2009). Developmental changes in cognitive control through adolescence. *Adv Child Dev Behav.* 37:233-78. Review.

Luna, B., Padmanabhan, A., & O'Hearn, K. (2010). What has fMRI told us about the development of cognitive control through adolescence? *Brain and Cognition, 72*(1), 101-113.

Lyng, G. D., Snyder-Keller, A., & Seegal, R. F. (2007). Polychlorinated biphenyl-induced neurotoxicity in organotypic cocultures of developing rat ventral mesencephalon and

striatum. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 97*(1), 128-139.

McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research, 146*(1-2), 97-103.

Marco, E. M., Macri, S., & Laviola, G. (2011). Critical age windows for neurodevelopmental psychiatric disorders: Evidence from animal models. *Neurotoxicity Research, 19*(2), 286-307.

Markham, J.A., Morris, J.R., & Juraska, J.M. (2007). Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. Neuroscience. 144(3):961-8.

Markham, J.A., Mullins, S.E., & Koenig, J.I. (2013). Periadolescent maturation of the prefrontal cortex is sex-specific and is disrupted by prenatal stress. *J Comp Neurol*.521(8):1828-43.

Mariussen E. & Fonnum F. (2001). The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. *Toxicology.* 159(1-2):11-21.

Mariussen, E., & Fonnum, F. (2006). Neurochemical targets and behavioral effects of organohalogen compounds: An update. *Critical Reviews in Toxicology, 36*(3), 253-289.

McGraw, J.E. & Waller, D.P. (2009). Fish ingestion and congener specific polychlorinated biphenyl and p,p'-dichlorodiphenyldichloroethylene serum concentrations in a great lakes cohort of pregnant African American women. *Environ Int*. 35(3):557-65

Neill, D.B. (1976). Frontal-striatal control of behavioral inhibition in the rat. *Brain Res*. 105(1):89-103. PubMed PMID: 1252961.

Newman, L. A., & McGaughy, J. (2011). Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Developmental Psychobiology, 53*(4), 391-401.

Newman, J., Gallo, M. V., Schell, L. M., DeCaprio, A. P., Denham, M., Deane, G. D., et al. (2009). Analysis of PCB congeners related to cognitive functioning in adolescents. *Neurotoxicology, 30*(4), 686-696.

Olesen, P.J., Nagy, Z., Westerberg, H., & Klingberg, T. (2003). Combined analysis of DTI and fMRI data reveals a joint maturation of white and grey matter in a fronto-parietal network. *Brain Res Cogn Brain Res.* 18(1):48-57.

Peters, B.D., Ikuta, T., DeRosse, P., John, M., Burdick, K.E., Gruner, P., Prendergast, D.M., Szeszko, P.R., & Malhotra, A.K. (2014). Age-related differences in white matter tract microstructure are associated with cognitive performance from childhood to adulthood. *Biol Psychiatry*. 75(3):248-56.

Ragozzino, M.E., Wilcox, C., Raso, M., & Kesner, R.P. (1999). Involvement of rodent prefrontal cortex subregions in strategy switching. *Behav Neurosci*. 113(1):32-41.

Rice, D. C. (1998). Effects of postnatal exposure of monkeys to a PCB mixture on spatial discrimination reversal and DRL performance. *Neurotoxicology and Teratology, 20*(4), 391-400.

Rice, D. C., & Hayward, S. (1997). Effects of postnatal exposure to a PCB mixture in monkeys on nonspatial discrimination reversal and delayed alternation performance. *Neurotoxicology, 18*(2), 479-494.

Richardson, J. R., & Miller, G. W. (2004). Acute exposure to aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. *Toxicology Letters, 148*(1-2), 29-40.

Richetto, J., & Riva, M. A. (2014). Prenatal maternal factors in the development of cognitive impairments in the offspring. *Journal of Reproductive Immunology,* 104-105: 20(5).

Ridderinkhof, K.R., van den Wildenberg, W.P., Segalowitz, S.J., & Carter, C.S. (2004). Neurocognitive mechanisms of cognitive control: the role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. *Brain Cogn*. 56(2):129-40. Review.

Ross, G. (2004). The public health implications of polychlorinated biphenyls (PCBs) in the environment. *Ecotoxicology and Environmental Safety, 59*(3), 275-291.

Rothmond DA, Weickert CS, Webster MJ. (2012). Developmental changes in human dopamine neurotransmission: cortical receptors and terminators. *BMC Neurosci*. 13:18.

Sable, H. J., Eubig, P. A., Powers, B. E., Wang, V. C., & Schantz, S. L. (2009). Developmental exposure to PCBs and/or MeHg: Effects on a differential reinforcement of low rates (DRL) operant task before and after amphetamine drug challenge. *Neurotoxicology and Teratology, 31*(3), 149-158.

Sable, H. J., Powers, B. E., Wang, V. C., Widholm, J. J., & Schantz, S. L. (2006). Alterations in
DRH and DRL performance in rats developmentally exposed to an environmental PCB
mixture. *Neurotoxicology and Teratology, 28*(5), 548-556.

Saint-Amour, D., Roy, M. S., Bastien, C., Ayotte, P., Dewailly, E., Despres, C., et al. (2006).
Alterations of visual evoked potentials in preschool inuit children exposed to
methylmercury and polychlorinated biphenyls from a marine
diet. *Neurotoxicology, 27*(4), 567-578.

Schaeffer, D. J., Dellinger, J. A., Needham, L. L., & Hansen, L. G. (2006). Serum PCB profiles
in native americans from wisconsin based on region, diet, age, and gender: Implications
for epidemiology studies. *The Science of the Total Environment, 357*(1-3), 74-87.

Seegal, R. F., Brosch, K. O., & Okoniewski, R. J. (2005). Coplanar PCB congeners increase
uterine weight and frontal cortical dopamine in the developing rat: Implications for
developmental neurotoxicity.*Toxicological Sciences : An Official Journal of the Society
of Toxicology, 86*(1), 125-131.

Seegal, R. F., Bush, B., & Brosch, K. O. (1985). Polychlorinated biphenyls induce regional
changes in brain norepinephrine concentrations in adult rats. *Neurotoxicology, 6*(3), 13-
23.

Seegal, R. F., Bush, B., & Brosch, K. O. (1991). Comparison of effects of aroclors 1016 and
1260 on non-human primate catecholamine function. *Toxicology, 66*(2), 145-163.

Seegal, R. F., Bush, B., & Brosch, K. O. (1991). Sub-chronic exposure of the adult rat to aroclor 1254 yields regionally-specific changes in central dopaminergic function. *Neurotoxicology, 12*(1), 55-65.

Seegal, R. F., Marek, K. L., Seibyl, J. P., Jennings, D. L., Molho, E. S., Higgins, D. S., et al. (2010). Occupational exposure to PCBs reduces striatal dopamine transporter densities only in women: A beta-CIT imaging study. *Neurobiology of Disease, 38*(2), 219-225.

Seegal, R. F., Okoniewski, R. J., Brosch, K. O., & Bemis, J. C. (2002). Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: An in vivo microdialysis study.*Environmental Health Perspectives, 110*(11), 1113-1117.

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Translational Psychiatry, 3*, e238.

Sjodin, A., Jones, R. S., Caudill, S. P., Wong, L. Y., Turner, W. E., & Calafat, A. M. (2014). Polybrominated diphenyl ethers, polychlorinated biphenyls, and persistent pesticides in serum from the national health and nutrition examination survey: 2003-2008. *Environmental Science & Technology, 48*(1), 753-760.

Sokolowski, J. D., & Salamone, J. D. (1994). Effects of dopamine depletions in the medial prefrontal cortex on DRL performance and motor activity in the rat. *Brain Research, 642*, 20-28.

Sowell, E.R., Thompson, P.M., Holmes, C.J., Jernigan, T.L., & Toga, A.W. (1999). In vivo

evidence for post-adolescent brain maturation in frontal and striatal regions. *Nat

Neuroscie.* 2(10):859-61.

Stevens MC, Kiehl KA, Pearlson GD, Calhoun VD. (2007). Functional neural networks

underlying response inhibition in adolescents and adults. *Behav Brain Res*. 181(1):12-22.

Stewart, P. W., Sargent, D. M., Reihman, J., Gump, B. B., Lonky, E., Darvill, T., et al. (2006).

Response inhibition during differential reinforcement of low rates (DRL) schedules may

be sensitive to low-level polychlorinated biphenyl, methylmercury, and lead exposure in

children. *Environmental Health Perspectives, 114*(12), 192301929.

Stewart, P., Fitzgerald, S., Reihman, J., Gump, B., Lonky, E., Darvill, T., et al. (2003). Prenatal

PCB exposure, the corpus callosum, and response inhibition. *Environmental Health

Perspectives, 111*(13), 1670-1677.

Stewart, P., Reihman, J., Gump, B., Lonky, E., Darvill, T., & Pagano, J. (2005). Response

inhibition at 8 and 9 1/2 years of age in children prenatally exposed to

PCBs. *Neurotoxicology and Teratology, 27*(6), 771-780.

Stewart, P. W., Reihman, J., Lonky, E. I., Darvill, T. J., & Pagano, J. (2003). Cognitive

development in preschool children prenatally exposed to PCBs and

MeHg. *Neurotoxicology and Teratology, 25*(1), 11-22.

Tao, G.Z. & Peterson, B.S. (2010). Normal development of brain circuits.

Neuropsychopharmacology. 35(1):147-68.

Teicher, M. H., Andersen, S. L., & Hostetter, J. C.,Jr. (1995). Evidence for dopamine receptor
    pruning between adolescence and adulthood in striatum but not nucleus
    accumbens. *Brain Research.Developmental Brain Research, 89*(2), 167-172.

Tunbridge, E. M., Bannerman, D. M., Sharp, T., & Harrison, P. J. (2004). Catechol-o-
    methyltransferase inhibition improves set-shifting performance and elevates stimulated
    dopamine release in the rat prefrontal cortex. *The Journal of Neuroscience : The Official
    Journal of the Society for Neuroscience, 24*(23), 5331-5335.

Turyk, M. E., Bhavsar, S. P., Bowerman, W., Boysen, E., Clark, M., Diamond, M., et al. (2012).
    Risks and benefits of consumption of great lakes fish. *Environmental Health
    Perspectives, 120*(1), 11-18.

Turyk, M., Anderson, H.A., Hanrahan, L.P., Falk, C., Steenport, D.N., Needham, L.L., Patterson,
    D.G. Jr, Freels, S., & Persky, V.; Great Lakes Consortium. (2006). Relationship of serum
    levels of individual PCB, dioxin, and furan congeners and DDE with Great Lakes sport-
    caught fish consumption. *Environ Res.* 100(2):173-83.

Wahlstrom, D., Collins, P., White, T., & Luciana, M. (2010). Developmental changes in
    dopamine neurotransmission in adolescence: behavioral implications and issues in
    assessment. Brain Cogn. 2010 Feb;72(1):146-59.

Wahlstrom, D., White, T., & Luciana, M. (2010). Neurobehavioral evidence for changes in
    dopamine system activity during adolescence. *Neurosci Biobehav Rev*. Apr;34(5):631-48.
    Review.

Webster, M.J., Elashoff, M., & Weickert, C.S. (2011). Molecular evidence that cortical synaptic

growth predominates during the first decade of life in humans. *Int J Dev Neurosci.*

29(3):225-36.

Weickert, C.S., Webster, M.J., Gondipalli, P., Rothmond, D., Fatula, R.J., Herman, M.M.,

Kleinman, J.E., & Akil, M. (2007). Postnatal alterations in dopaminergic markers in the

human prefrontal cortex. *Neuroscience.* 144(3):1109-19.

Widholm, J. J., Clarkson, G. B., Strupp, B. J., Crofton, K. M., Seegal, R. F., & Schantz, S. L.

(2001). Spatial reversal learning in aroclor 1254-exposed rats: Sex-specific deficits in

associative ability and inhibitory control. *Toxicology and Applied Pharmacology, 174*,

188-198.

Widholm, J. J., Villareal, S., Seegal, R. F., & Schantz, S. L. (2004). Spatial alternation deficits

following developmental exposure to aroclor 1254 and/or methylmercury in

rats. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 82*(2),

577-589.

Yetnikoff, L., Reichard, R. A., Schwartz, Z. M., Parsely, K. P., & Zahm, D. S. (2014).

Protracted maturation of forebrain afferent connections of the ventral tegmental area in

the rat. *Journal of Comparative Neurology,522*(5), 1031-1047.

Chapter 2: Specific Aims

As reviewed in Chapter 1, there is a large body of research describing polychlorinated biphenyls (PCBs) as ubiquitous environmental contaminants that are neurotoxic if exposure occurs perinatally – a critical period in brain development. Both human and animal studies have suggested that PCBs are associated with deficits in executive functions such as response inhibition, working memory and cognitive flexibility (Crinnion, 2011; Eubig *et al,* 2010; Korrick & Sagiv, 2008). These deficits are thought to be mediated by dysfunction of neurochemical systems of the brain, notably the dopamine (DA) system (Eubig *et al,* 2010). Adolescence is another critical period of brain development in which the frontal lobes and neurochemical systems therein undergo prominent remodeling and maturation. More specifically, the prefrontal cortex (PFC) and dopaminergic innervation of the PFC are undergoing marked maturation during this time (reviewed in Spear, 2013 and Selemon, 2013). Thus, executive functions mediated by the PFC, including response inhibition and cognitive flexibility, may be especially sensitive to disruption during adolescence. Given that perinatal PCB exposure seems to target both executive functioning and dopamine neurochemistry, it is likely that PCB exposure during adolescence could adversely impact brain and behavior. However, little research has been done examining the potential for adverse effects of PCB exposure during this critical period of brain development. The goal of this research project is to fill this important gap in scientific research. Moreover, this research question will be addressed using parallel human and animal studies. The animal study was designed to closely model the cognitive endpoints tested in a cohort of adolescents exposed to PCBs through fish consumption. Human studies are, by necessity, correlational; thus, consistent findings in human epidemiological studies and carefully designed and controlled animal studies are important to increase certainty that effects observed in humans are the result

of the PCB exposure and not due to other uncontrolled variables. Because human populations are not exposed to individual PCB congeners in isolation, the animal study described herein used an environmentally relevant PCB mixture formulated to mimic the PCB congener profile found in walleye in the Fox River in northeastern WI, a body of water from which the human cohort consumed sport-caught fish. The animal study allows assessment of a potential mechanism underlying PCB-induced cognitive deficits: dopamine system dysfunction. Together, the results of the human and animal studies outlined in this proposal will provide valuable insight into the effects of adolescent exposure to PCBs on brain and behavior.

Aim 1: Determine if exposure to PCBs during adolescence will alter performance on tasks of executive function that engage the PFC. Previous research in our lab has shown that early developmental exposure to PCBs can lead to deficits in higher-order cognitive functions in humans and animals. Executive functions such as cognitive flexibility, working memory, attention and response inhibition are sensitive to perinatal PCB exposure and mediated largely by the PFC. Therefore, the current studies examined the effects of PCB exposure during a period of robust prefrontal cortical development – adolescence. **It was hypothesized that rats exposed to PCBs during adolescence would show impaired performance in adulthood on tasks of cognitive flexibility (set-shifting) and response inhibition (differential reinforcement of low rates of responding) as compared to non-exposed controls.** To test this hypothesis, female Long-Evans rats were mated and pups weaned at PND 21. Three male and three female pups from each litter were then retained for dosing and subsequent cognitive testing. One male and one female pup from each litter were randomly assigned to one of three treatment groups: 0, 3 and 6 mg PCB/kg/day of the Fox River PCB mixture. Dosing, via oral exposure, began at PND 27 and continued daily through PND 50 to capture the whole period of adolescence in rats. At

around PND 90, rats began operant testing on a set-shifting task that assesses cognitive flexibility. Upon completion (around PND 110), these rats moved on to a differential reinforcement of low rates of responding (DRL) operant task used to assess response inhibition.

Aim 2: Assess whether PCB exposure during adolescence alters levels of dopamine transporter expression using Western blot analysis. Both of the operant tasks described above have been shown to be sensitive to disruption of the mesocortical dopamine pathway, and evidence suggests that PCBs can lead to disruption of the brain DA system. Specifically, studies have found a PCB-induced reduction in dopamine transporter (DAT) expression in rat brain tissue that may be due in part to permanent DA cell loss in brain regions containing these cell populations. Thus, disrupted DA function may be the mechanism underlying cognitive deficits seen with early-life PCB exposure. There are no previous animal studies that have assessed long-term changes in DAT expression after early PCB exposure, but long-lasting changes in DAT expression have been seen with exposure to other environmental influences such as atrazine (Li *et al,* 2014) and social stress (Novick *et al,* 2011) during critical periods of early development. Many studies have found the medial prefrontal cortex (mPFC) to be involved in response inhibition and cognitive set-shifting and the orbital frontal cortex (OFC) to be involved in reversal learning (another aspect of cognitive flexibility that was tested in our set shifting task). Thus, punches of the OFC and mPFC were collected for western blot analysis of DAT expression once these rats completed cognitive testing. Striatal punches were also collected because previous studies have found PCB-induced reduction in DAT expression in this brain region (Richardson & Miller, 2004; Caudle *et al,* 2006). **It was hypothesized that rats exposed to PCBs during adolescence would show a reduction in DAT expression lasting into adulthood as compared to non-exposed controls.**

Aim 3: Assess the association of PCB exposure with performance on tasks of cognitive flexibility and response inhibition in a cohort of adolescent children of sports anglers in Green Bay, Wisconsin. In this study, data were collected from a cohort of about 115 12-18 year old children of sports anglers in Green Bay, Wisconsin where the population is exposed to PCBs through fish consumption. Blood PCB levels were measured in subjects at the time of assessment. The Conners' Parent Report was used as a measure of cognitive problems and inattention, oppositional behavior, hyperactivity and ADHD. The Cambridge Neuropsychological Test Automated Battery (CANTAB) Intradimensional/ Extradimensional Set-Shifting task (ID/ED) was used to look at cognitive flexibility in this cohort. Response inhibition was also assessed in these adolescents using the Integrated Visual and Auditory Continuous Performance Task (IVA). Health history and diet information were collected. **It was hypothesized that higher blood PCB concentrations in these adolescents would be associated with higher (worse) scores in the Conners' parent report subscales that measure cognitive problems/inattention and ADHD, poorer performance on the ID/ED set-shifting task and poorer performance on the IVA continuous performance task.**

<div align="center">References</div>

Crinnion, W. J. (2011). Polychlorinated biphenyls: Persistent pollutants with immunological, neurological, and endocrinological consequences. *Alternative Medicine Review: A Journal of Clinical Therapeutic, 16*(1), 5-13.

Eubig, P. A., Aguiar, A., & Schantz, S. L. (2010). Lead and PCBs as risk factors for attention Deficit/Hyperactivity disorder. *Environmental Health Perspectives, 118*(12), 1654-1667.

Korrick, S. A., & Sagiv, S. K. (2008). Polychlorinated biphenyls, organochlorine pesticides and neurodevelopment. *Current Opinion in Pediatrics, 20*(2), 198-204.

Li, Y., Sun, Y., Yang, J., Wu, Y., Yu, J., et al. (2014). Age-dependent dopaminergic dysfunction following fetal exposure to atrazine in SD rats. *Environ Toxicol Pharmacol*. 37(3):1275-82.

Novick, A.M., Forster, G.L., Tejani-Butt, S.M., Watt, M.J. (2011). Adolescent social defeat alters markers of adult dopaminergic function. *Brain Res Bull.* 86(1-2):123-8.

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Translational Psychiatry, 3*, e238.

Spear, L.P. (2013). Adolescent Neurodevelopment. *Journal of Adolescent Health.* 52(2):S7-13.

Chapter 3: Cognitive Set-Shifting and Response Inhibition in Adult Rats Exposed to an Environmental PCB Mixture during Adolescence

Abstract

Converging evidence from studies in animal models and humans suggests that early developmental exposure to polychlorinated biphenyls (PCBs) leads to deficits in cognitive flexibility and inhibitory control. These processes are mediated to a large extent by the prefrontal cortex, thus we examined the effects of PCB exposure during adolescence—a period of robust prefrontal cortical development—on both processes. The study used operant set-shifting and differential reinforcement of low rates of responding (DRL) tasks to assess cognitive flexibility and response inhibition, respectively. As PCBs have been shown previously to reduce dopamine transporter (DAT) expression, DAT expression was also quantified in relevant brain regions. One male and one female pup from each of 14 litters were assigned to each of three treatment groups: 0, 3 or 6 mg PCB/kg/day. Rats were dosed orally from postnatal day (PND) 27-50 to capture the whole period of adolescence in rats. At approximately PND 90, they began testing in the set-shifting task which included an initial visual cue discrimination, an extra-dimensional shift to a position discrimination and a reversal of the position discrimination. There were no group differences in trials to criterion on visual cue discrimination or on the shift from visual to position discrimination in either males or females. During the position reversal, the 3 and 6 mg/kg males took significantly fewer trials to reach criterion than control males. No group differences were observed in females. These results suggest a male-specific effect of adolescent PCB-exposure on the reversal phase of the set shifting task. Following set-shifting, rats moved on to DRL in which they were required to withhold responding for a specified period of time (15 seconds) in order to receive a reinforcer. There were no exposure-related group differences in

total presses or efficiency ratio in males or in females. Western blot analysis revealed no significant group differences in DAT protein expression in the orbital frontal cortex (OFC) or striatum in males or females. In summary, there were subtle sex-specific effects of adolescent PCB exposure on the reversal phase of the set-shifting task but no effects of exposure on performance on the DRL 15 task. DAT expression in relevant brain areas was not altered due to PCB exposure.

Introduction

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants formerly used as lubricants and dielectric fluids in capacitors and transformers as well as in the production of carbonless copy paper, caulking material and fluorescent light ballasts (Ross, 2004). PCBs are also inadvertently produced as a byproduct of the manufacture of paint pigments and thus continue to be found in commercially available products that ultimately terminate in landfills and adjacent ecosystems (Grossman, 2013). Furthermore, older buildings still containing PCBs in caulking and fluorescent light ballasts will continue to contribute PCBs to ecosystems as they are remediated or demolished (Hornbuckle & Robertson, 2010). Thus, PCBs are likely to continue to persist in our environment for the foreseeable future.

PCBs can cross the placenta and can be released into breast milk during lactation (Jacobson *et al*, 1984), and because of this the potential health effects of perinatal exposure to PCBs have been the topic of much research over the last four decades. Developmental PCB exposure has been associated with impairments of executive function in humans and animals (reviewed in Eubig *et al*, 2010). In particular, deficits in cognitive flexibility have been seen in rats and monkeys perinatally exposed to PCBs (reviewed in Sable & Schantz, 2006). Response inhibition is also disrupted in rats (Sable *et al,* 2009), monkeys (Rice & Hayward, 1997; Rice, 1998) and children (Stewart *et al,* 2006) developmentally exposed to PCBs.

Research has shown that executive functions such as cognitive flexibility and response inhibition are mediated to a large extent by the prefrontal cortex (PFC) (reviewed in Dalley *et al*, 2004). Experimental inactivation or ablation of areas of the PFC causes deficits in tasks engaging these aspects of executive function (Floresco *et al*, 2006; McAlonan & Brown, 2003), and dopamine (DA) depletion in the PFC leads to similar deficits (Roberts *et al,* 1994; Sokolowski &

Salamone, 1994), suggesting an important role for PFC DA in mediating cognitive flexibility and response inhibition.

Both *in vitro* and *in vivo* studies have reported DA system dysfunction following PCB exposure. Studies have found reductions in DA concentrations in cell cultures as well as in the striatum of rats (Seegal *et al,* 2002) and non-human primates (Seegal *et al,* 1991) following exposure to PCBs. DA decreases in the striatum have also been reported in weanling rats perinatally exposed to PCBs (Castoldi *et al,* 2006). More in line with deficits in executive functions seen in PCB-exposed humans and animals, one study found that rats perinatally exposed to an ortho-substituted PCB congener showed a reduction in frontal cortical DA levels that persisted into adulthood (Seegal *et al,* 2005). Other studies have reported permanent reductions in DA neuron number due to adult exposure to PCBs in the substantia nigra and ventral midbrain, including the ventral tegmental area, the site of DA cell bodies that project to the prefrontal cortex (Lee *et al*, 2012; reviewed in Fonnum & Mariussen, 2009). This evidence in combination with the PCB-induced reduction in DA concentrations suggests that PCB exposure could lead to hypofunction of the DA system in the prefrontal cortex.

Expression of both the dopamine transporter (DAT), responsible for reuptake of the DA in the synaptic cleft, and the vesicular monoamine transporter (VMAT2), responsible for repackaging cytosolic DA into vesicles for later release, is inhibited by PCB exposure (Seegal *et al,* 2010; Bemis & Seegal, 2004; Caudle *et al,* 2006; Fonnum *et al,* 2006; Lyng *et al,* 2007; Richardson & Miller, 2004). The effects of PCBs on DAT and VMAT2 expression likely contribute to the reduction in DA levels reported in numerous studies. PCB exposure has also been found to alter concentrations of other neurochemicals such as norepinephrine, serotonin, glutamate, and GABA, but the effects on the DA system have been the most consistently studied

and replicated (Mariussen & Fonnum, 2001; Seegal *et al*, 1985). Of note, our lab previously reported that perinatally PCB-exposed rats given amphetamine, a psychostimulant with known effects through DAT (reviewed in Gowrishankar *et al*, 2014), showed reduced locomotor sensitization to amphetamine (Poon *et al*, 2013) and less disrupted performance on differential reinforcement of low rates of responding (DRL) – an operant task of response inhibition (Sable *et al*, 2009). Taken together, these studies suggest that hypofunction of the DA system may underlie some of the deficits in cognitive function seen with early PCB exposure.

Although extensive research has been carried out evaluating the effects of perinatal PCB exposure on cognitive functioning in humans and animals, very little research has assessed the effects of PCB exposure during another critical period of brain development: adolescence. During this period, the frontal lobes are undergoing marked plasticity and maturation. Specifically, a great deal of synaptic remodeling is occurring, and dopaminergic innervation of the PFC is being refined (reviewed in Lenroot *et al*, 2007; Marco *et al*, 2011; Selemon, 2013). These changes likely underlie the cognitive improvements that emerge during or after adolescence (Brenhouse & Andersen, 2011). For instance, adult rats perform better than adolescent rats on tasks engaging the prefrontal cortex, including tests of response inhibition (Andrezejewski *et al*, 2011) and behavioral set-shifting (Newman & McGaughy, 2011). In humans, performance on tasks of response inhibition is poor in childhood but steadily improves with age, reaching adult levels of performance in mid to late adolescence (Luna *et al*, 2010). Thus, this critical period of frontal lobe development and maturation may be especially vulnerable to environmental influences, including exposure to environmental neurotoxicants.

Given the previous research indicating that perinatal PCB exposure results in deficits in response inhibition and cognitive flexibility (reviewed in Eubig *et al*, 2010) and the further

development of these cognitive abilities that is occurring during adolescence, it was hypothesized that adolescence would be another critical period when PCB exposure could result in deficits in these aspects of executive function. To address this question, operant tests of set shifting and response inhibition (DRL) were administered to adult rats exposed to an environmentally relevant PCB mixture throughout adolescence. Because decreased DAT expression and DA dysfunction associated with PCB exposure may underlie deficits in executive functions seen in exposed humans and animals (Eubig *et al,* 2010), we sought to determine whether adolescent PCB exposure led to changes in DAT expression in the brains of these animals in adulthood. There is evidence, albeit scarce, that exposure to environmental stressors imposed during critical periods of brain development (i.e., the prenatal, early postnatal and adolescent periods) can lead to long-lasting changes in DAT expression. For instance, rats perinatally exposed to atrazine, an herbicide and known neurotoxicant, led to a reduction in DAT expression in the substantia nigra 6 months after exposure (Li *et al,* 2014). Another study found that social stress during adolescence increased DAT density in the medial PFC of brains sampled several weeks after the stress ended (Novick *et al,* 2011).

Most previous PCB studies have used individual PCB congeners or commercial PCB mixtures, but these approaches do not accurately represent what is in the environment and what human populations are exposed to. In this study, we used an experimental PCB mixture formulated to mimic the PCB congener profile found in walleye (a popular sport-caught fish) in the Fox River in northeastern WI, a body of water from which the human cohort we have also studied consumed sport-caught fish (Kostyniak *et al,* 2005).

Methods

Animals

Twenty-one nulliparous female and 21 male Long-Evans rats, approximately 70 days of age, were purchased from Harlan (Madison, WI). Animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Rats were individually housed in standard plastic shoebox cages with beta-chip (virgin hardwood) bedding, in a temperature- and humidity-controlled room (22°C, 40-55% humidity) and were maintained on a 12-hour reverse light-dark cycle (lights off at 0830 h). Standard rat chow and water were available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.*

Exposure

Male and female rats were individually paired for breeding for 8 days. Only litters with 7 pups or greater were kept, and larger litters were culled to 8-10 pups per litter on postnatal day (PND) 2. At weaning (PND 21), 3 male and 3 female pups from each litter were retained for cognitive testing. One male and 1 female pup from each litter were randomly assigned to each of 3 treatment groups: 0, 3 and 6 mg/kg/day PCBs (n=14, n=13, and n=14 male-female littermate pairs, respectively). The PCB mixture used in this study was formulated to mimic the congener profile found in walleye, taken from the Fox River in northeast Wisconsin, thereby closely mimicking human PCB exposure from fish consumption. The mixture consisted of 35% Aroclor 1242 (Monsanto Lot KB 05-415), 35% Aroclor 1248 (AccuStandards Lot F-110), 15% Aroclor 1254 (Monsanto Lot KB 05-612), and 15% Aroclor 1260 (AccuStandards Lot 021-020). The mixture was found to have relatively low aryl hydrocarbon receptor (AhR) activity, but high

ryanodine receptor (RyR) activity (Kostyniak *et al*, 2005). The chemicals were dissolved in corn oil to yield the dosing solutions.

Dosing began at PND 27 and continued daily through PND 50 to capture the whole period of adolescence in both female and male rats. The doses were chosen based on previous work done with perinatal exposure to PCBs in our lab. Offspring of dams given these doses daily through gestation and lactation show deficits in inhibitory control (Sable *et al*, 2009). Pups were weighed daily through the dosing period, doses were adjusted daily to account for weight gain, and the appropriate amount of dosing solution was pipetted directly into the mouth of the pup. Beginning on PND 90, rats were weighed daily and access to food was restricted to 85% of the rats' free-feeding weight in order to keep the animals motivated to work for food rewards in the operant chambers. Food restriction has been routinely used in our lab, and there is no evidence that it confounds PCB-mediated effects.

Apparatus

Behavioral testing was conducted in 24 automated operant chambers (Med Associates; St. Albans, VT) housed in sound attenuated cubicles, each ventilated by a fan. All operant chambers contained a stimulus cue lamp above each of the two retractable response levers, which were located symmetrically on both sides of the pellet trough approximately 5.5 centimeters above the floor. A white-noise generator masked extraneous sounds, and a sonalert speaker was used to signal reinforcement. The experimental contingencies were programmed using Medstate Notation behavioral programming language (Med Associates; St. Albans, VT).

Procedure

Set-Shifting

Programs described herein for the set-shifting task were modified from programs shared with us by Stan Floresco (Floresco *et al*, 2008; Butts *et al,* 2013).

Pretraining

Rats were first trained to lever press using a fixed ratio (FR) 1 schedule of reinforcement in which a reward was delivered for each single lever press. During this pretraining phase, cue lights above the levers were not illuminated and one lever was presented and remained extended during the entire session. The rat had to make 50 presses in a session in order to progress to the next phase. After the rat successfully completed the first phase of FR1, the next phase required the rat to make 50 presses in one session on the opposite lever. Once the rat successfully completed this phase, it moved on to retractable lever press training for 5 sessions. In this phase, one of the two levers extended. The lever retracted once a response was made; after a 20 second inter-trial interval, one of the two levers extended again. The rat had to press the lever within 10 seconds of its insertion; otherwise, the lever retracted and the trial was counted as an "omission". Rats received 90 such trials in a daily session. Immediately following the last session of retractable lever press training (in the same day), side bias was determined for each rat. In this short session, stimulus lights above the levers were not illuminated, and both levers were inserted into the chamber. This phase of training started with both levers extended. A press on either of the levers resulted in the delivery of a food pellet and both levers retracting. Twenty seconds later, both levers were extended again; if the rat chose the same lever as before, the levers retracted without dispensing a pellet. This continued until the rat made a press on the opposite lever. Thus, the trial did not end until both levers were pressed. The program continued until 7 trials were completed. The rat's side preference was determined by where the majority of first lever choices (left or right lever) were made.

Visual Cue Discrimination

After pretraining was completed, rats were trained on visual-cue discrimination where illumination of the stimulus light predicted reward. Each trial in this session was initiated with the illumination of one of the stimulus lights located above either the right or left lever. Then, the house light turned on, and both levers were extended into the chamber. The rat then had 10 seconds to respond. A press on the lever below the illuminated cue light resulted in the delivery of one reward pellet and retraction of both levers. If a rat failed to respond within 10 seconds or responded on the incorrect lever, no reward pellet was delivered and both levers retracted. Twenty seconds later the levers were again extended. This continued for 160 trials per session. To move on to the next phase, the rat had to reach a criterion of 65% correct in one session.

Position discrimination (Set-shift)

Once rats reached criterion in visual-cue discrimination, they began the strategy-shift phase of the experiment where they had to disengage from the previously learned visual-cue strategy and shift to a new egocentric response strategy that now predicted reward. A rat's ability to shift from a previously relevant strategy to a new strategy is an index of its cognitive flexibility. The position discrimination strategy required the rat to press the lever opposite its side bias in order to obtain reward. The lit cue light no longer predicted reward and was now an irrelevant dimension. To evaluate how well the rat remembered what it had learned previously, in the first 20 trials of this session rats continued to be reinforced for pressing the lever associated with the lit cue light. Beginning with the 21st trial, the lever opposite the rat's side bias was reinforced, forcing the animal to shift to a new strategy in order to obtain a food reinforcer. After a response was made, the levers retracted. Twenty seconds later the levers were re-extended. This continued for 160 trials per session. Like the visual cue discrimination phase,

sessions continued until the rat reached a criterion of 65% correct in a session.

Reversal Learning

After they reached criterion on position discrimination, rats moved on to the position reversal. In this phase, rats were required to respond on the lever opposite that rewarded during the initial position discrimination in order to earn a reinforcer. Visual cues still served as distracters during this phase. After a response was made, the levers retracted. Twenty seconds later the levers were re-extended. This continued for 160 trials. Reversal learning was tested in only one session.

*Differential Reinforcement of Low Rates of Responding (DRL) train*ing

After rats completed the whole set-shifting battery, they were tested on a DRL schedule. During DRL testing only the right lever was used and it remained extended during the entire test session. During the first phase, a 1 second inter-response time (IRT) (DRL 1) was required in order to obtain a reinforcer. The first training phase lasted for 2 sessions regardless of performance. During the second and third phases, the IRT required for reinforcement was increased to 5 seconds (DRL 5) for 2 sessions and then 10 seconds (DRL 10) for 2 sessions. During each training phase animals were rewarded for the first lever press occurring after the specified time interval had elapsed. Responses occurring before the required IRT had elapsed reset the timer, requiring the animal to wait another full interval before a response would result in reinforcement. All training sessions terminated after 200 reinforcers were delivered or 90 minutes had elapsed, whichever occurred first.

DRL testing

Following DRL training, rats were given 30 daily sessions that required a 15 second IRT in order to obtain a reinforcer (DRL 15). Similar to the training phases, the first response after 15

seconds had elapsed resulted in a reinforcer. Responses made before the 15 seconds elapsed reset the timer and delayed reinforcement. Daily sessions terminated after 200 reinforcers were delivered or 90 min had elapsed, whichever occurred first. After 30 sessions on DRL 15, rats moved on to DRL extinction for 3 days in which rats were no longer reinforced for lever presses. Each daily session terminated after 90 minutes.

Western Blot Analysis

Brain Dissection

Upon completion of operant testing, rats were sacrificed and brains collected for western blot analysis (~ PND150). Slices were taken on a coronal plane from the anterior to posterior direction using a microtome with a frozen stage (-20°C). Slices were removed until Bregma 5.20 mm was reached, at which point the sizes of the frontal cortices and the olfactory bulbs were approximately equal. Then a 1.75 mm (females) or 1.85 mm (males) thick coronal slice was cut. The slice was placed on a glass slide with the anterior side facing down. Bilateral punches of the orbital frontal cortex (OFC) were taken from these sections using a 2 mm biopsy punch (Harris Uni-Core; Ted Pella Inc, CA). Then, slices were removed until Bregma 1.70 mm was reached, at which point the two sides of the corpus callosum were < 1 mm apart. From this point, a 1.75 mm (females) or 2.0 mm slice (males) thick coronal slice was cut and placed on a glass slide with the posterior side facing down. Bilateral punches of the striatum were taken from these sections using the 2 mm biopsy punch.

Protein Extraction and Quantitation

Tissue punches were homogenized in a 375μL mixture of TPER Tissue Protein Extraction Reagent (Thermo Scientific) and Complete Mini Protease Inhibitor (PI) Cocktail Tablets (Roche). Homogenized samples were centrifuged at 10,000×g for 5 minutes, and the

supernatant was used in the Pierce bicinchoninic acid (BCA) protein assay (Thermo Scientific).

Also at this time, an aliquot of the supernatant from each sample was diluted to 1:20 to be loaded

onto NuPAGE Novex 4-12% Bis-Tris Gels for western blot analysis. The BCA protein assay

was performed using the directions for the microplate procedure provided in the instructions of

the Pierce BCA Protein Assay Kit. For each sample, 10μL of protein supernatant was diluted in

15 μL of TPER-PI so that the measured protein amounts were within the range of the standard

curve. The samples and standards were added to wells of a microplate and incubated at 37˚ C for

30 minutes. The absorbance of the samples at 562 nm was then measured using a Multiskan

Ascent microplate reader (Type 354; Thermo Scientific). Protein concentrations were calculated

using Ascent Software (v. 2.6, Revision 3.1, Dec. 2003; Thermo Scientific).

In order to compare protein expression across different gels, a protein standard was

created by preparing tissue (using the procedure described above) from the mPFC, OFC and

NAc from one non-exposed subject and combining the supernatant from the 3 samples. As with

all of the other samples, a 1:20 dilution of the protein was used for western blot analysis, and the

protein standard was loaded into one lane of each gel. For each sample, a volume equal to a

target amount of 0.25 μg diluted protein was added to reducing agent, sample buffer, and T-

PER/PI to attain a sample volume of 15 μL. For the molecular weight ladder, 5 μL of marker was

used in place of 0.25 μg protein. Samples were denatured by heating at 70˚ C for 10 minutes.

Then the protein ladder, protein standard, and protein samples were loaded into the lanes of the

gel. Each gel contained samples from one brain region and at least one male and one female

sample from each treatment group. Once running buffer was added, gels were electrophoresed at

150 volts for 1 hour. After electrophoresis, gels were then transferred onto Invitrolon PVDF

membranes (0.45 µM pore size) and electrophoresed in transfer buffer in a cold room at 30V for 1.5 hours.

Blocking and Probing

Following gel transfer, PVDF membranes were incubated in Tris-buffered saline with 5% nonfat dry blocking milk for 1 hour. Then, the membranes were incubated overnight at 4ºC in an anti-dopamine transporter rabbit polyclonal antibody to the C-terminus of DAT (#AB2231; Millipore) at 1:2000 w/v in Tris-buffered saline with 5% nonfat dry milk. The following day, membranes were incubated in goat anti-rabbit secondary antibody (#12-348; Millipore) at 1:2000 w/v in 1% nonfat dry milk for 1 hr. Membranes were then treated with LumiGLO reagent (Cell Signaling Technologies) for one minute, and chemiluminescence was captured using x-ray film. Membranes were then stripped for 20 min with Restore Plus Western Blot Stripping Buffer (Thermo Scientific) and incubated overnight at 4ºC with a rabbit monoclonal α-tubulin antibody (Abcam ab4074) at 1:10,000 w/v in Tris-buffered saline with 2% nonfat dry milk. α-Tubulin antibody binding was detected using goat anti-rabbit secondary antibody (Ab6721) at 1:2500 w/v, and the film developing process was repeated. α-Tubulin blots were used to ensure equal protein loading across samples. Following probing for DAT, bands were detected between the 76K and 102K molecular weight markers (at about 80 kDa), which was the anticipated location for DAT. Following probing for alpha tubulin, bands were detected above the 52K molecular weight marker (at about 55 kDa), which was the anticipated location for alpha tubulin. Films were then manually scanned into a computer and DAT and α-tubulin band densities measured using image J software (version 1.46r; http://imagej.nih.gov/ij). DAT and alpha tubulin band densities were determined and then standardized to the protein standard densities on each image

to obtain relative densities for DAT and alpha tubulin. Adjusted densities for the samples were determined by dividing the relative density of each sample by the relative density of the standard.

Statistical Analyses

Data are reported as mean ± SEM. All statistical analyses were conducted using SPSS for MS Windows (version 22.0, SPSS Inc.; Chicago, IL) with statistical significance set at $p<0.05$. Because of previously reported sex differences in PCB-related effects on set-shifting (Widholm *et al*, 2001), and DRL (Sable et al., 2009) male and female data were analyzed separately. In the interest of brevity, only significant exposure-related main effects and interactions are reported. Additional post hoc analyses were conducted as appropriate to determine the nature of significant effects that were detected via the initial omnibus analyses.

Visual Cue Discrimination, Shift to Position discrimination, Response Reversal

Group differences in the number of trials to criterion on each measure were determined via univariate ANOVA with exposure (0, 3 or 6 mg/kg) as a between-subjects variable and litter as a covariate.

DRL

Group differences in total number of lever presses and ratio of reinforced:non-reinforced responses from DRL1, DRL5 and DRL10 were analyzed in 3 separate ANOVAs using 3 (exposure) x 2 (day) mixed ANOVAs with testing day as a repeated measures factor and litter as a covariate. For DRL15, data were averaged across five-day blocks to yield 6 testing blocks. The primary measures assessed were total presses and the ratio of reinforced:non-reinforced responses (efficiency ratio). These dependent measures were analyzed separately using a 3 (exposure) x 6 (block) mixed ANOVA with testing block as a repeated measures factor and litter as a covariate. Each response made during DRL15 was also categorized into one of eight 2.5

second inter-response time (IRT) bins. The proportion of responses falling within each IRT bin was calculated and averaged across the 5 days in the first testing block (acquisition) and sixth testing block (steady state). Each of these was analyzed separately using a 3 (exposure) x 8 (bin) mixed ANOVA with IRT bin as a repeated measures factor and litter as a covariate.

Western Blot Analysis

Relative densities adjusted for the protein standard (as described above) were analyzed in the OFC and striatum. Adjusted DAT densities were analyzed separately for each of these regions using one-way ANOVAs with exposure as the between-subjects factor.

Results

Visual Cue Discrimination

Mean trials to criterion for visual cue discrimination are represented in Figure 1. In males, there was no significant difference across exposures for trials to criterion although males in the 6 mg/kg PCB exposure group did appear to take more trials to reach criterion than the other two groups. There was also no significant difference in trials to criterion across exposures in females with females of all exposure groups performing similarly throughout this phase. Litter did not contribute significantly to the statistical model.

Shift to Position discrimination

Mean trials to criterion in the position discrimination phase are presented in Figure 2. There was no significant main effect of exposure in males in this phase with all exposure groups taking a similar number of trials to reach criterion. Similarly, there was no significant main effect of exposure in females in this phase with all exposure groups taking a similar number of trials to reach criterion. Litter did not contribute significantly to the statistical model.

Response Reversal

Mean trials to criterion for the response reversal phase are presented in Figure 3. A significant effect of exposure was found in males [$F(2,6.860)$, p=0.003]. Post-hoc analysis (Tukey HSD) revealed significant differences between males of the 0 mg/kg and 3 mg/kg groups (p=0.023) and also between males of the 0 mg/kg and 6 mg/kg groups (p=0.003), with males of the 0 mg/kg group taking more trials to reach criterion than males in both the 3 and 6 mg/kg groups. Males of the 3 mg/kg group and the 6 mg/kg group did not differ significantly from one another. No significant differences in performance were observed across exposures in the females.

DRL 1, DRL5, DRL10

For total presses in DRL 1 (data not shown), there were no significant main effects of exposure or session in males or in females nor was there a significant session by exposure interaction in males or females. Thus, performance did not differ across sessions, and there were no exposure-related differences in total presses across sessions. There were also no significant main effects of treatment or session in males or females in efficiency ratio in DRL 1. Similar to total presses, performance did not differ across sessions, and efficiency ratio was not affected by exposure. Litter did not contribute to the statistical model for total presses or efficiency ratio in DRL 1.

For total presses in DRL 5 (data not shown), there was no significant main effect of exposure, but there were significant main effects of session in males [$F(1, 12.230)$, p=0.001] and in females [$F(1, 30.794)$, p<0.0001] which was expected as performance improved (i.e., total presses decreased) across sessions. There was no significant session by exposure interaction in males or females suggesting that there were no exposure-related differences in total presses across DRL 5 sessions. There was no significant main effect of exposure on efficiency ratio

54

during DRL 5, but again, there were significant main effects of session in males [$F_{(1, 24.075)}$, p<0.0001] and females [$F_{(1, 26.174)}$, p<0.0001] with the efficiency ratio improving (i.e., increasing) across sessions. There was no significant session by exposure interaction on efficiency ratio in males or in females indicating that performance across sessions did not differ due to exposure. Litter did not contribute to the statistical model for total presses or efficiency ratio in DRL 5.

Total presses in DRL 10 for males and females are presented in Figure 4 (4A and 4B, respectively). There was no significant main effect of session or exposure in males, but there was a significant session x exposure interaction [$F_{(2, 4.095)}$, p=0.025]. Post-hoc analysis (Tukey HSD), revealed a significant difference in total presses across sessions in males of the 3 mg/kg group (p=0.021). This effect resulted from the fact that the decline in total presses in the 3 mg/kg males across these 2 sessions was steeper than in the other two groups. The 0 mg/kg and 6 mg/kg males showed no significant difference in total presses across sessions. Litter did not contribute significantly to this model. Females showed a similar pattern of results for total presses in DRL 10. There was no significant main effect of session or exposure in females, but there was a significant session x exposure interaction [$F_{(2, 5.264)}$, p=0.010]. However, unlike in the males, post-hoc analysis showed no significant differences between groups across sessions. Litter did contribute significantly to this model (p=0.026). Efficiency ratios for males and females are presented in Figure 4 (4C and 4D, respectively). There was no significant main effect of session or exposure in males, and there was no significant session x exposure interaction. Thus, males of all groups were performing with a similar pattern of efficiency across sessions. In females, there was no significant main effect of exposure, but as with total presses there was a significant session x exposure interaction [$F_{(2, 3.801)}$, p=0.032]. However, as with total presses, post-hoc

analysis showed no significant differences between groups across. In females, litter contributed significantly to this model (p=0.028).

DRL15

Total presses in DRL 15 are represented in Figure 5A for males and females. There was no significant main effect of exposure on total presses in males or in females, but there was a significant effect of block on total presses in both males [$F(5,4.862)$, p<0.0001] and females [$F(5,19.955)$, p<0.0001]. This was expected, with all groups improving their performance across blocks. There was no significant block x exposure interaction. This was true in males and females. Litter did not contribute significantly to these models.

Figure 5B presents the efficiency ratios (reinforced:non-reinforced) for males and females. There was no significant main effect of exposure on efficiency ratio in males or in females, but there was a significant effect of block on efficiency ratio in both males [$F(5,11.801)$, p<0.0001] and females [$F(5,15.543)$, p<0.0001]. Again, this was expected with all groups improving their performance across blocks. There was no significant block x exposure. Litter did not contribute significantly to these models.

Figure 6 depicts IRT bins for males and females in Block 1 (Panels A and B) and Block 6 (Panels C and D). Analysis of IRT bins in Block 1 (Days 1-5) revealed no significant exposure by bin interaction in males or females suggesting that response patterns across the 15 second interval were not different across the three exposure groups in either males or females. In block 1, there was a significant effect of bin in males [$F(7,3.672)$, p=0.001] and in females [$F(7,9.924)$, p<0.0001] which was expected as response patterns change across the 15 second interval (Figures 6A and 6B). In Block 6 (Days 26-30), there was no significant bin by exposure interaction in males or in females suggesting that the proportion of responses falling within each

bin were not different due to exposure. There was a significant effect of bin in males

[$F(7,10.068)$, p<0.0001] and in females [$F(7,14.582)$, p<0.0001]. As expected, there was a

bursting pattern of response in the first 2.5 seconds followed by a reduction in responses over the

next bins, with responses gradually increasing over the rest of the interval (Figures 6C and 6D).

DRL EXT

There was no significant main effect of exposure on total presses during DRL extinction

(Figure 7). Performance was similar for the three exposure groups in both males and females.

There was a significant effect of session that was expected as males [$F(2, 38.459)$, p<0.0001]

and females [$F(2, 45.119)$, p<0.0001] of all exposure groups were making fewer presses across

days on extinction. There was no significant session by exposure interaction suggesting that total

presses did not differ across sessions due to exposure. Litter did not contribute significantly to

the statistical model.

Western Blot Analysis

Figure 8 represents an example of western blots for DAT expression in the OFC (Panel

A) and striatum (Panel B). There was no significant main effect of exposure on DAT protein

expression in the OFC of males or females (Figure 9A). Similarly, there was no significant main

effect of exposure on DAT protein expression in the striatum of males or of females (Figure 9B).

Discussion

In this study, we assessed cognitive flexibility, response inhibition and DAT expression

in adult male and female rats exposed to PCBs during adolescence. In the set-shifting task, we

saw a sex-specific effect of PCBs on reversal learning. In the DRL task, we saw a subtle effect of

PCBs in both males and females during the last training phase of the task (DRL 10). The PCB-

related effects in performance on these two cognitive tasks were not as robust as hypothesized,

and, perhaps not surprisingly then, there were no significant differences in DAT expression in the OFC or the striatum.

Set-shifting

The results on the set-shifting task were unexpected. Adolescent PCB exposure did not impair learning of the extra-dimensional shift as was hypothesized, and the PCB-exposed males actually acquired the position reversal more readily than controls. Although these results were contrary to our hypothesis, they do suggest that adolescent PCB exposure may be disrupting the cognitive processes involved in reversal learning. Interestingly, a recent study found a similar pattern of performance on the same operant set-shifting task in rats exposed to acute stress (Thai *et al,* 2013). In that study there was no significant difference in performance due to stress on the visual cue discrimination or the position discrimination. In the position reversal phase, however, stressed rats took fewer trials to reach criterion than controls. Thus, as was seen in our study, acute stress seemed to facilitate performance on reversal learning. The authors conjectured that acute stress may have biased the rats toward a strategy other than the spatial strategy that should have been acquired in the position discrimination phase. The reversal learning phase of the task required the rats to press the lever that they previously demonstrated to be biased toward pressing during the earlier side preference test. Learning the position discrimination and the reversal would normally engage spatial response learning (i.e., learning that the lever located in one position in the operant box was associated with the reward), but because the reversal phase involved pressing on an already favored-lever, it is possible that stressed (or PCB-exposed) rats may revert to an unextinguished habit over learning a new strategy. These results may indicate the possibility that our adolescent PCB-exposed rats did not form a response set to the spatial

strategy during the position discrimination phase and were able to more quickly revert to habit (i.e., their lever-bias) to perform the reversal in fewer trials than the controls.

DRL

In the DRL task, we saw no significant effect of PCB exposure on performance on DRL15, contrary to our hypothesis. These DRL findings are inconsistent with what has been found with early postnatal PCB exposure in monkeys. Although the monkey studies did report deficits on DRL performance associated with PCB exposure (Rice 1998; Rice 1999), these studies used a PCB mixture with a different congener make up than the Fox River mixture used here. The monkeys were also exposed in the early postnatal period and not as adolescents. Furthermore, these studies used a DRL 30 in which monkeys had to withhold responses for 30 seconds in order to earn a reinforcer. It is possible that deficits in DRL performance would emerge in the PCB exposed rats in our study if they were required to withhold responding for a longer period of time.

The lack of an effect of exposure on DRL 15 was not completely unexpected, however, because PCB-related deficits in performance have not been consistent across studies. In one study using DRL15, rats exposed perinatally to the Fox River mixture showed a lower ratio of reinforced to nonreinforced responses suggesting an impairment on this task (Sable *et al,* 2009). However, an earlier study found no significant effect of perinatal PCB treatment on DRL 15 performance (Sable *et al,* 2006). These studies used the same PCB mixture and doses; however, an important difference between these two studies was that the rats that showed a deficit in performance were only tested on the DRL task, whereas in the study that did not see an effect of exposure rats were tested on another operant task just prior to DRL testing. Thus, it is possible that a transfer of experience occurred such that rats exposed to another operant task prior to DRL

testing tend to perform the task more efficiently than rats for which the DRL task was their first exposure to operant training. In the current study, our rats were tested on another operant task (set-shifting) prior to DRL testing, and this prior experience could have made it harder to detect an effect on the DRL task.

DAT Measures

Despite a male-specific exposure-related difference in performance on reversal learning, there was no significant difference in DAT expression in the OFC or the striatum across PCB exposure groups in either males or females (Figure 8). Several studies have reported long-lasting changes in DAT expression due to environmental factors present during critical developmental windows (Novick *et al,* 2011; Li *et al,* 2014), thus we hypothesized that PCBs given throughout adolescence could lead to long lasting changes in DAT. In line with this hypothesis, a previous study did observe a decrease in PFC DA concentrations that persisted into adulthood following early PCB exposure (Seegal *et al,* 2005). However, given that the results from our cognitive tasks were subtle and not in the direction we hypothesized, it is not surprising that we did not observe long-term changes in DAT expression due to chronic PCB exposure during adolescence.

Conclusions

In this study we reported a sex-specific effect of PCB exposure on an operant set-shifting task where exposed males showed better performance on reversal learning compared to controls, but we saw no effect of exposure on a DRL task of response inhibition or DAT expression in related brain regions. Although the findings of this study did not support our hypotheses as expected, there were some differences in cognition associated with adolescent PCB exposure that suggest there may be vulnerability to PCBs during this period. In the future, it would be useful to explore PCB-related effects on other executive functions mediated by the PFC, such as working

memory given that research has suggested PCB-related deficits on tasks of working memory in both humans and animals (reviewed in Eubig *et al,* 2010). Adolescence is a period of multidimensional growth and maturation. As individuals are exploring their environments more independently, innumerable environmental influences are shaping brain, cognition and behavior, thus making research on the effects of neurotoxicants such as PCBs and other environmental factors a valuable contribution to our understanding of this critical period of development.

Figures

Figure 1: Visual Cue Discrimination
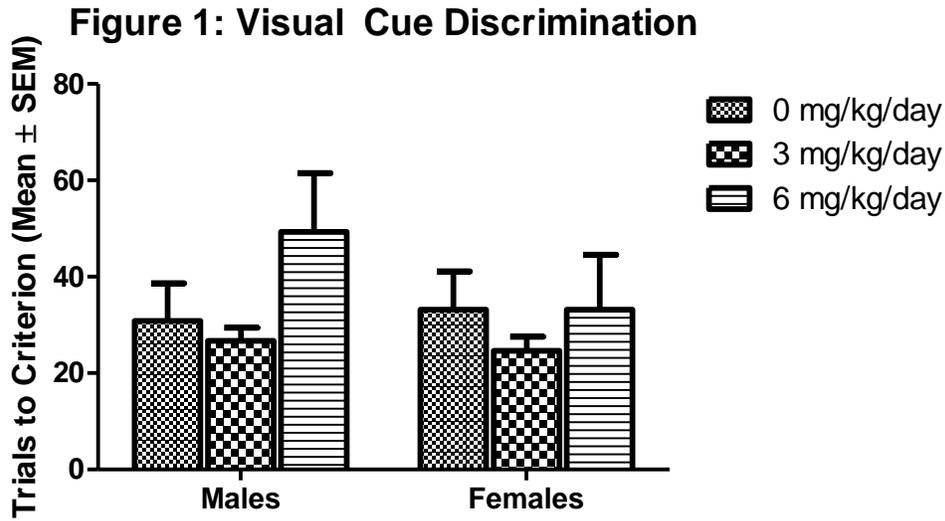
Figure 1: Effects of PCB exposure on trials to criterion in visual cue discrimination. There were no significant differences in performance across exposures in males or in females.

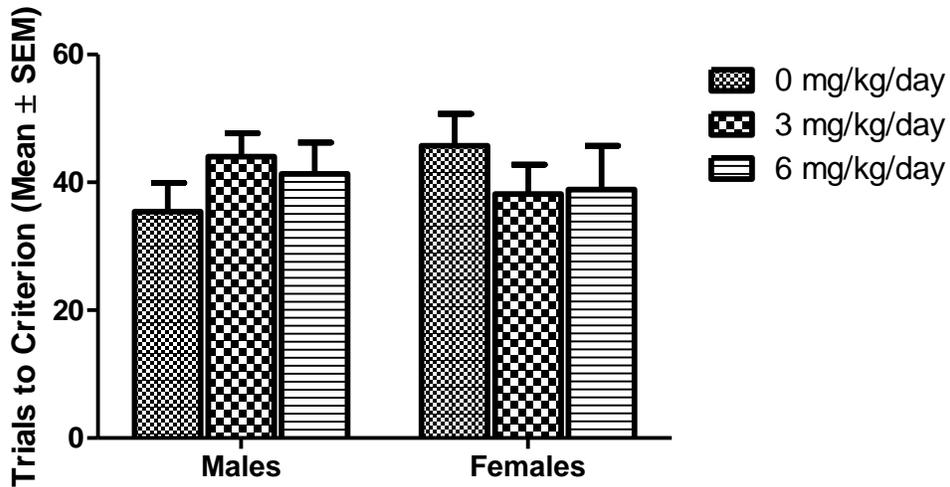## Figure 2: Response Discrimination (Set-Shift)

Figure 2: Effects of PCB exposure on trials to criterion in position discrimination. There were no significant differences in performance across exposures in males or in females.
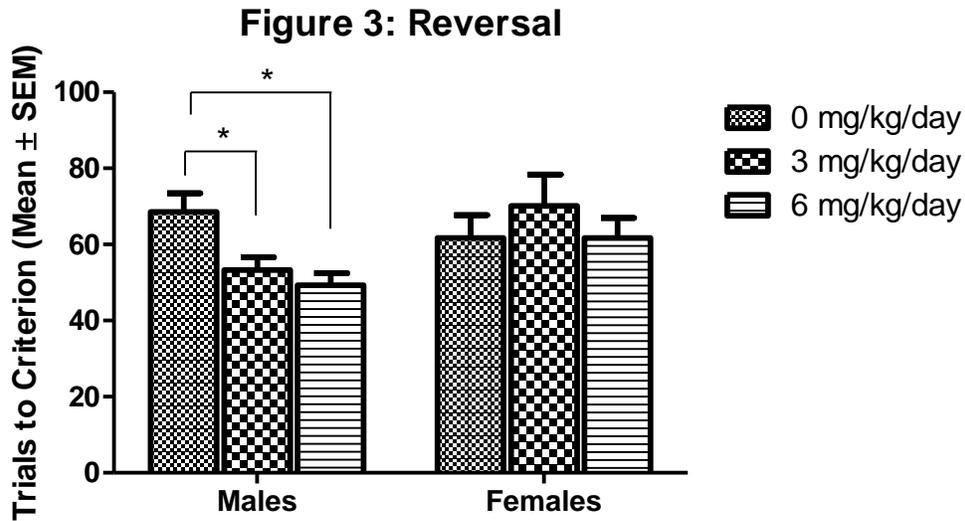
Figure 3: Effects of PCB exposure on trials to criterion in the reversal phase. Males of the 0 mg/kg group took more trials to reach criterion than males of the 3 mg/kg group (p=0.023) and males of the 6 mg/kg group (p=0.003). No significant difference in performance was observed across groups in females.
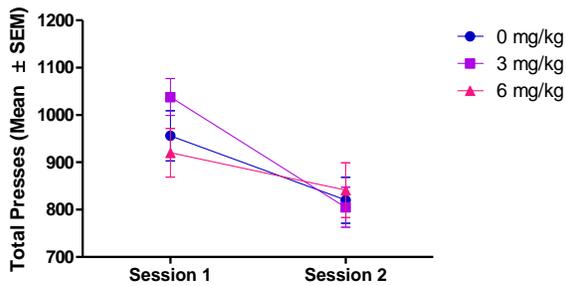

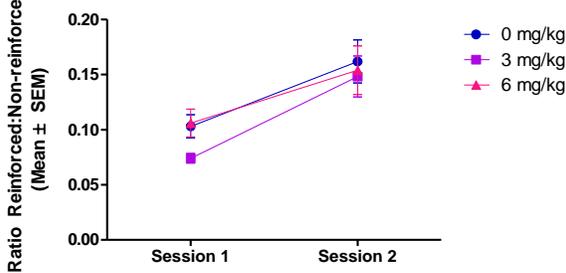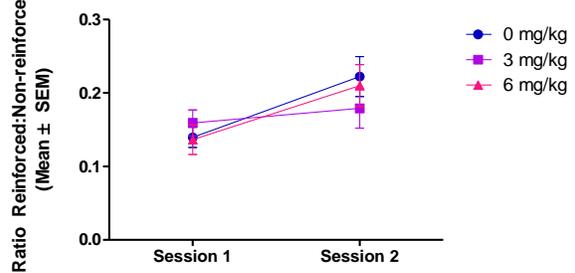Figure 4: Effects of PCB exposure on DRL10 total presses and efficiency ratio. For total presses, there was no significant main effect of exposure in males (Panel A), but there was a significant session x exposure interaction (p=0.025). Post-hoc analysis revealed a significant difference in total presses across sessions in males of the 3 mg/kg group (p=0.021). The 0 mg/kg and 6 mg/kg males showed no significant difference in total presses across sessions. Females showed a similar pattern of results for total presses in DRL 10 (Panel B). There was no significant main effect of exposure in females. There was a significant session x exposure interaction (p=0.010), but the post-hoc analyses were not significant. For efficiency

ratio, there was no significant main effect of exposure or a significant session x exposure interaction in males (Panel C). In females, there was no significant main effect of exposure. There was a significant session x exposure interaction (p=0.032) (Panel D), but the post-hoc analyses were not significant.

Figure 5A: DRL15 Total Presses

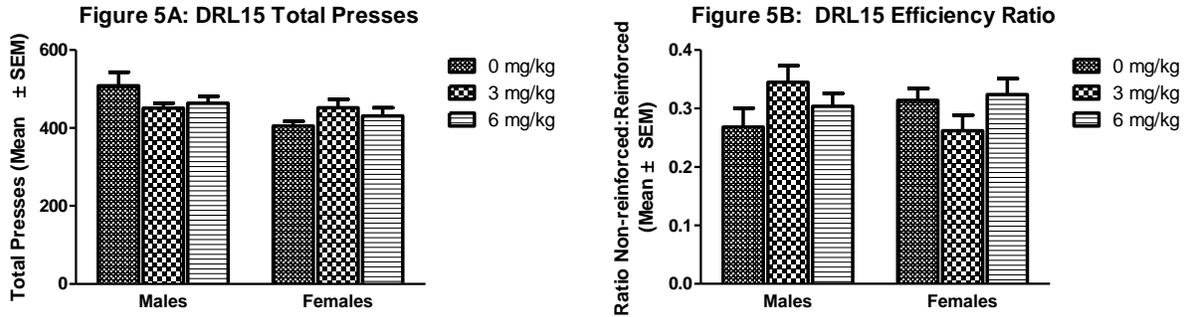**Figure 5B: DRL15 Efficiency Ratio**

Figure 5: Effects of PCB exposure on total presses and efficiency ratio in DRL 15. There was no significant effect of exposure on total presses in males or in females (Panel A). There was no significant main effect of exposure on efficiency ratio in males or in females (Panel B).

Figure 6A: Block 1 IRTs (Males)

Figure 6B: Block 1 IRTs (Females)

Figure 6C: Block 6 IRTs (Males)
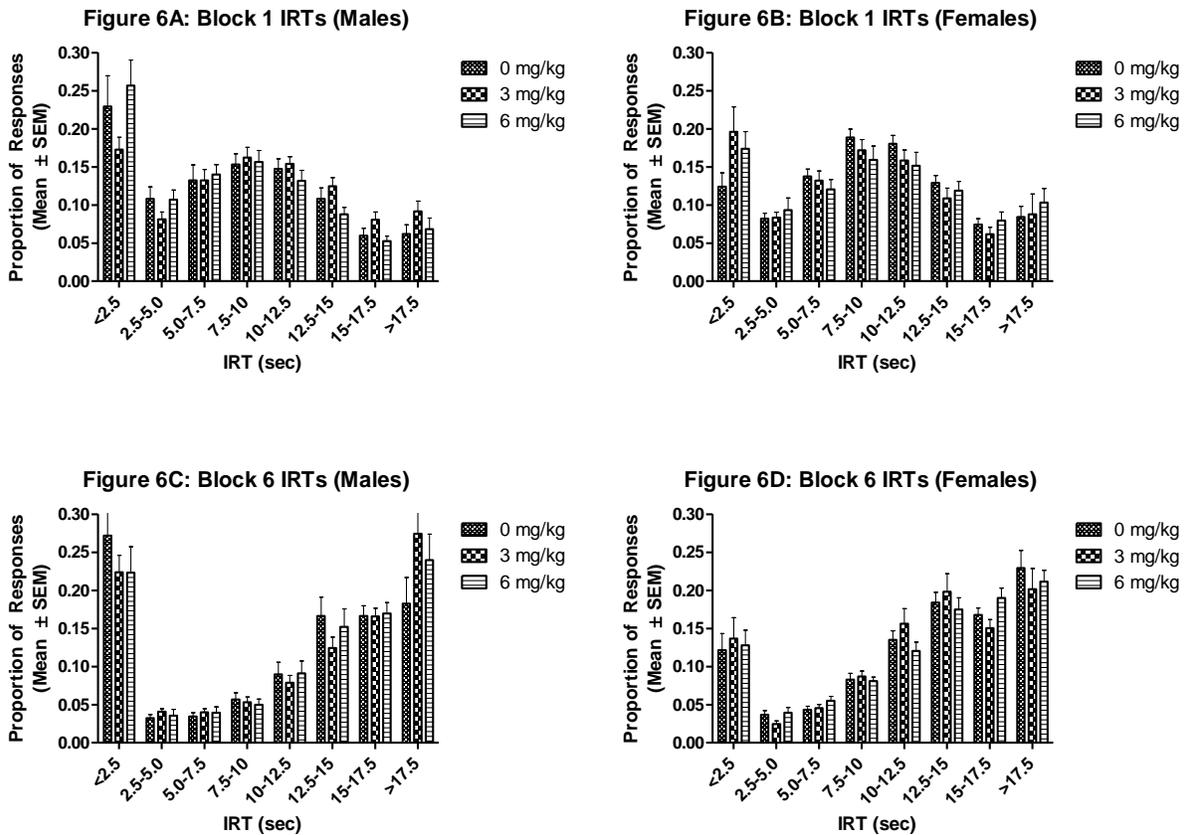
Figure 6D: Block 6 IRTs (Females)

Figure 6: DRL 15 IRT bins. In block 1, there was no significant exposure by bin interaction in males (Panel A) or females (Panel B) suggesting that response patterns across the 15 second interval were not different across the three exposure groups in either sex. There was a significant effect of

bin in males (p=0.001) and in females (p<0.0001) as response patterns change across the 15 second interval. In block 6, there was no significant exposure by bin interaction in males (Panel C) or in females (Panel D) as response patterns across bins were not different across groups in either sex. There was a significant effect of bin in males (p<0.0001) and in females (p<0.0001) as expected with a bursting pattern of response in the first 2.5 seconds followed by a reduction in responses that gradually increase over the rest of the interval.

Figure 7: DRL EXT Total Presses
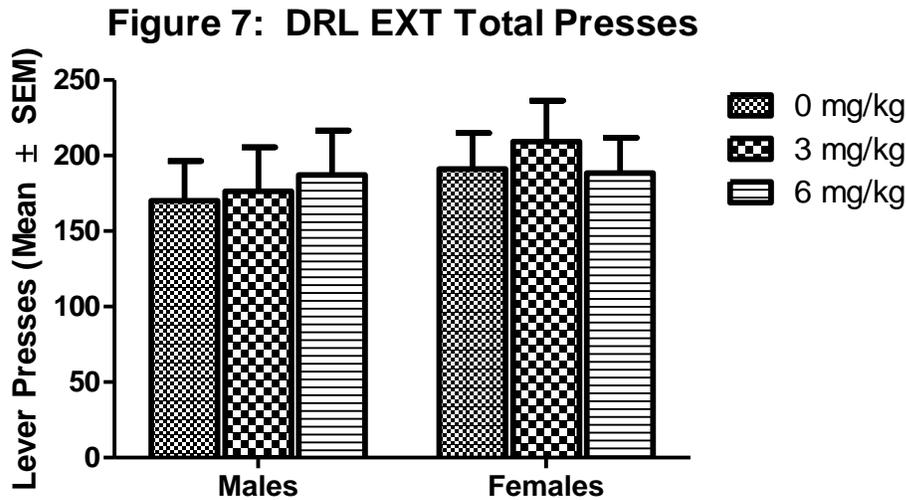
Figure 7: Total lever presses across males and females during DRL extinction. There were no significant differences in mean lever presses across exposure groups in males or in females as all groups made fewer lever presses each day on extinction.
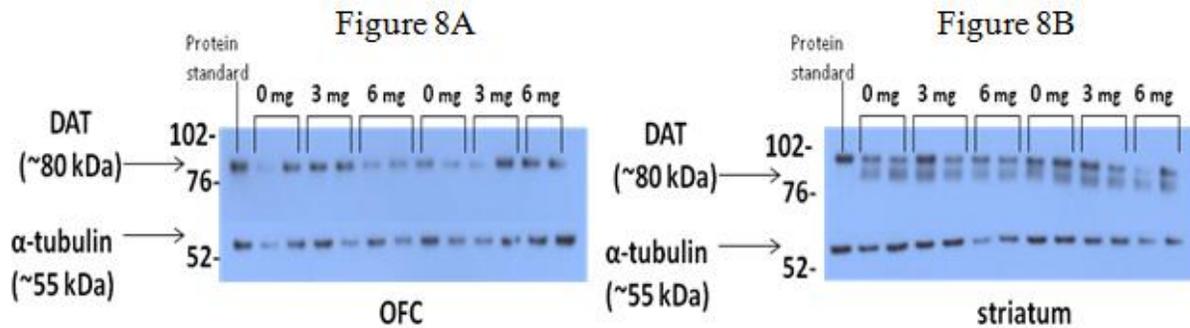
Figure 8: Sample western blots for DAT expression in the OFC (Panel A) and the striatum (Panel B). DAT expression is detected around 80 kDa. Alpha-tubulin expression is detected around 55 kDa. DAT and alpha tubulin band densities were determined and then standardized to the protein standard densities on each image to obtain relative densities for DAT and alpha tubulin.

Figure 9A: DAT Expression in OFC   **Figure 9B: DAT Expression in striatum**

Figure 9: DAT relative density in the OFC and striatum. There was no significant difference in DAT expression in the OFC across exposure groups in males or in females (Panel A). Similarly, there was no significant difference in DAT expression in the striatum across exposure groups in males or in females (Panel B).

References

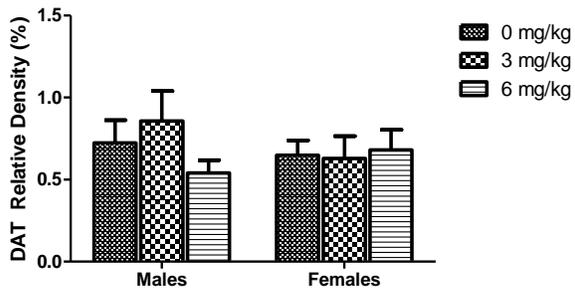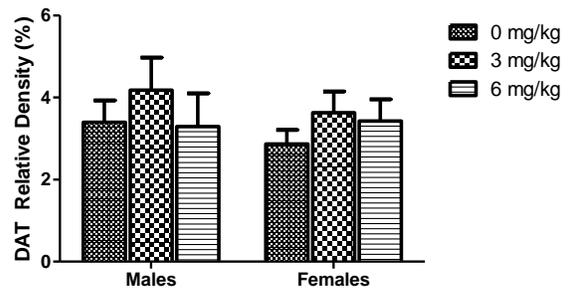Andrzejewski, M. E., Schochet, T. L., Feit, E. C., Harris, R., McKee, B. L., & Kelley, A. E. (2011). A comparison of adult and adolescent rat behavior in operant learning, extinction, and behavioral inhibition paradigms. *Behavioral Neuroscience, 125*(1), 93-105.

Bava, S., & Tapert, S. F. (2010). Adolescent brain development and the risk for alcohol and other drug problems. *Neuropsychology Review, 20*(4), 398-413.

Bemis, J. C., & Seegal, R. F. (2004). PCB-induced inhibition of the vesicular monoamine transporter predicts reductions in synaptosomal dopamine content. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 80*(2), 288-295.

Brenhouse, H.C. & Andersen, S.L. (2011).Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. *Neurosci Biobehav Rev.* 35(8):1687-703.

Butts, K. A., Floresco, S. B., & Phillips, A. G. (2013). Acute stress impairs set-shifting but not reversal learning. *Behavioural Brain Research, 252*, 222-229.

Castoldi, A. F., Blandini, F., Randine, G., Samuele, A., Manzo, L., & Coccini, T. (2006). Brain monoaminergic neurotransmission parameters in weanling rats after perinatal exposure to methylmercury and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153). *Brain Research,* 1112(1), 91-98.

Caudle, W. M., Richardson, J. R., Delea, K. C., Guillot, T. S., Wang, M., Pennell, K. D., et al. (2006). Polychlorinated biphenyl-induced reduction of dopamine transporter expression as

a precursor to parkinson's disease-associated dopamine toxicity. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 92*(2), 490-499.

Crinnion, W. J. (2011). Polychlorinated biphenyls: Persistent pollutants with immunological, neurological, and endocrinological consequences. *Alternative Medicine Review : A Journal of Clinical Therapeutic,* 16(1), 5-13.

Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2004). Prefrontal executive and cognitive functions in rodents: Neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews, 28*(7), 771-784.

Eubig, P. A., Aguiar, A., & Schantz, S. L. (2010). Lead and PCBs as risk factors for attention Deficit/Hyperactivity disorder. *Environmental Health Perspectives, 118*(12), 1654-1667.

Floresco, S. B., Block, A. E., & Tse, M. T. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural Brain Research, 190*(1), 85-96.

Floresco, S. B., Magyar, O., Ghods-Sharifi, S., Vexelman, C., & Tse, M. T. (2006). Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 31*(2), 297-309.

Fonnum, F., Mariussen, E., & Reistad, T. (2006). Molecular mechanisms involved in the toxic effects of polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs). *J Toxicol Environ Health A*. 2006 Jan 8;69(1-2):21-35.

Fonnum, F., & Mariussen, E. (2009). Mechanisms involved in the neurotoxic effects of

    environmental toxicants such as polychlorinated biphenyls and brominated flame

    retardants. *Journal of Neurochemistry, 111*(6), 1327-1347.

Gowrishankar, R., Hahn, M.K., & Blakely, R.D. (2014). Good riddance to dopamine: roles for

    the dopamine transporter in synaptic function and dopamine-associated brain disorders.

    Neurochem Int. 73:42-8.

Grossman E. (2013). Nonlegacy PCBs: pigment manufacturing by-products get a second look.

    Environ Health Perspect. 121(3):A86-93.

Gulley, J. M., & Juraska, J. M. (2013). The effects of abused drugs on adolescent development of

    corticolimbic circuitry and behavior. *Neuroscience, 249*, 3-20.

Hankosky, E. R., & Gulley, J. M. (2013). Performance on an impulse control task is altered in

    adult rats exposed to amphetamine during adolescence. *Developmental

    Psychobiology, 55*(7), 733-744.

Hornbuckle, K. & Robertson, L. (2010). Polychlorinated biphenyls (PCBs): sources, exposures,

    toxicities. *Environ Sci Technol*. 44(8):2749-51.

Jacobson, J. L., Fein, G. G., Jacobson, S. W., Schwartz, P. M., & Dowler, J. K. (1984). The

    transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across

    the human placenta and into maternal milk. *American Journal of Public Health, 74*(4), 378-

    379.

Juraska, J. M., Sisk, C. L., & DonCarlos, L. L. (2013). Sexual differentiation of the adolescent

   rodent brain: Hormonal influences and developmental mechanisms. *Hormones and*

   Behavior, 64(2), 203-210.

Kostyniak, P. J., Hansen, L. G., Widholm, J. J., Fitzpatrick, R. D., Olson, J. R., Helferich, J. L.,

   et al. (2005). Formulation and characterization of an experimental PCB mixture designed to

   mimic human exposure from contaminated fish. *Toxicological Sciences : An Official*

   Journal of the Society of Toxicology, 88(2), 400-411.

Lee, D. W., Notter, S. A., Thiruchelvam, M., Dever, D. P., Fitzpatrick, R., Kostyniak, P. J., et al.

   (2012). Subchronic polychlorinated biphenyl (aroclor 1254) exposure produces oxidative

   damage and neuronal death of ventral midbrain dopaminergic systems.*Toxicological*

   Sciences : An Official Journal of the Society of Toxicology, 125(2), 496-508.

Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, et al. (2007). Sexual

   dimorphism of brain developmental trajectories during childhood and adolescence.

   Neuroimage. 36(4):1065-73.

Li Y, Sun Y, Yang J, Wu Y, Yu J, et al. (2014). Age-dependent dopaminergic dysfunction

   following fetal exposure to atrazine in SD rats. *Environ Toxicol Pharmacol*. 37(3):1275-

   82.

Lilienthal, H., Heikkinen, P., Andersson, P. L., & Viluksela, M. (2013). Sexually dimorphic

   behavior after developmental exposure to characterize endocrine-mediated effects of

   different non-dioxin-like PCBs in rats. *Toxicology, 311*(1-2), 52-60.

Luna, B., Padmanabhan, A., & O'Hearn, K. (2010). What has fMRI told us about the development of cognitive control through adolescence? *Brain and Cognition, 72*(1), 101-113.

Lyng, G. D., Snyder-Keller, A., & Seegal, R. F. (2007). Polychlorinated biphenyl-induced neurotoxicity in organotypic cocultures of developing rat ventral mesencephalon and striatum. *Toxicological Sciences : An Official Journal of the Society of Toxicology, 97*(1), 128-139.

Marco, E. M., Macri, S., & Laviola, G. (2011). Critical age windows for neurodevelopmental psychiatric disorders: Evidence from animal models. *Neurotoxicity Research, 19*(2), 286-307.

Mariussen E, Fonnum F. (2001). The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. *Toxicology.* 159(1-2):11-21.

Mariussen, E., & Fonnum, F. (2006). Neurochemical targets and behavioral effects of organohalogen compounds: An update. *Critical Reviews in Toxicology, 36*(3), 253-289.

McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research, 146*(1-2), 97-103.

Newman, L. A., & McGaughy, J. (2011). Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Developmental Psychobiology, 53*(4), 391-401.

Novick AM, Forster GL, Tejani-Butt SM, Watt MJ. (2011). Adolescent social defeat alters

markers of adult dopaminergic function. *Brain Res Bull.* 86(1-2):123-8.

Poon, E., Monaikul, S., Kostyniak, P. J., Chi, L. H., Schantz, S. L., & Sable, H. J. (2013).

Developmental exposure to polychlorinated biphenyls reduces amphetamine behavioral

sensitization in long-evans rats. *Neurotoxicology and Teratology, 38*, 6-12.

Rice, D. C. (1997). Effect of postnatal exposure to a PCB mixture in monkeys on multiple fixed

interval-fixed ratio performance. *Neurotoxicology and Teratology, 19*(6), 429-434.

Rice, D. C. (1998). Effects of postnatal exposure of monkeys to a PCB mixture on spatial

discrimination reversal and DRL performance. *Neurotoxicology and Teratology, 20*(4),

391-400.

Rice, D. C. (1999). Behavioral impairment produced by low-level postnatal PCB exposure in

monkeys. *Environmental Research, 80*(2 Pt 2), S113-S121.

Rice, D. C. (1999). Effect of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout

gestation and lactation on development and spatial delayed alternation performance in

rats. *Neurotoxicology and Teratology, 21*(1), 59-69.

Rice, D. C., & Hayward, S. (1997). Effects of postnatal exposure to a PCB mixture in monkeys

on nonspatial discrimination reversal and delayed alternation

performance. *Neurotoxicology, 18*(2), 479-494.

Richardson, J. R., & Miller, G. W. (2004). Acute exposure to aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. *Toxicology Letters, 148*(1-2), 29-40.

Roberts, A. C., De Salvia, M. A., Wilkinson, L. S., Collins, P., Muir, J. L., Everitt, B. J., et al. (1994). 6-hydroxydopamine lesions of the prefrontal cortex in monkeys enhance performance on an analog of the wisconsin card sort test: Possible interactions with subcortical dopamine. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 14*(5 Pt 1), 2531-2544.

Rogan, W. J., & Brown, S. M. (1979). Some fundamental aspects of epidemiology: A guide for laboratory scientists. *Federation Proceedings, 38*(5), 1875-1879.

Ross, G. (2004). The public health implications of polychlorinated biphenyls (PCBs) in the environment. *Ecotoxicology and Environmental Safety, 59*(3), 275-291.

Sable, H. J., Eubig, P. A., Powers, B. E., Wang, V. C., & Schantz, S. L. (2009). Developmental exposure to PCBs and/or MeHg: Effects on a differential reinforcement of low rates (DRL) operant task before and after amphetamine drug challenge. *Neurotoxicology and Teratology, 31*(3), 149-158.

Sable, H. J., Monaikul, S., Poon, E., Eubig, P. A., & Schantz, S. L. (2011). Discriminative stimulus effects of cocaine and amphetamine in rats following developmental exposure to polychlorinated biphenyls (PCBs). *Neurotoxicology and Teratology, 33*(2), 255-262.

Sable, H. J., Powers, B. E., Wang, V. C., Widholm, J. J., & Schantz, S. L. (2006). Alterations in
DRH and DRL performance in rats developmentally exposed to an environmental PCB
mixture. *Neurotoxicology and Teratology, 28*(5), 548-556.

Sable, H. J. K., & Schantz, S. L. (2006). Executive function following developmental exposure
to polychlorinated biphenyls (PCBs): What animal models have told us. In E. D. Levin, &
J. J. Buccafusco (Eds.), *Animal models of cognitive impairment*. Boca Raton (FL): Taylor &
Francis Group, LLC.

Schantz, S. L., Levin, E. D., Bowman, R. E., Heironimus, M. P., & Laughlin, N. K. (1989).
Effects of perinatal PCB exposure on discrimination-reversal learning in
monkeys. *Neurotoxicology and Teratology, 11*(3), 243-250.

Seegal, R. F., Brosch, K. O., & Okoniewski, R. J. (1997). Effects of *in utero* and lactational
exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine
function. *Toxicology and Applied Pharmacology, 146*, 95-103.

Seegal, R. F., Brosch, K. O., & Okoniewski, R. J. (2005). Coplanar PCB congeners increase
uterine weight and frontal cortical dopamine in the developing rat: Implications for
developmental neurotoxicity. *Toxicological Sciences : An Official Journal of the Society of
Toxicology, 86*(1), 125-131.

Seegal, R. F., Bush, B., & Brosch, K. O. (1985). Polychlorinated biphenyls induce regional
changes in brain norepinephrine concentrations in adult rats. *Neurotoxicology, 6*(3), 13-23.

Seegal, R. F., Bush, B., & Brosch, K. O. (1991). Comparison of effects of aroclors 1016 and 1260 on non-human primate catecholamine function. *Toxicology, 66*(2), 145-163.

Seegal, R. F., Bush, B., & Brosch, K. O. (1991). Sub-chronic exposure of the adult rat to aroclor 1254 yields regionally-specific changes in central dopaminergic function. *Neurotoxicology, 12*(1), 55-65.

Seegal, R. F., Marek, K. L., Seibyl, J. P., Jennings, D. L., Molho, E. S., Higgins, D. S., et al. (2010). Occupational exposure to PCBs reduces striatal dopamine transporter densities only in women: A beta-CIT imaging study. *Neurobiology of Disease, 38*(2), 219-225.

Seegal, R. F., Okoniewski, R. J., Brosch, K. O., & Bemis, J. C. (2002). Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: An in vivo microdialysis study. *Environmental Health Perspectives, 110*(11), 1113-1117.

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Translational Psychiatry, 3*, e238.

Sherrill, L. K., Stanis, J. J., & Gulley, J. M. (2013). Age-dependent effects of repeated amphetamine exposure on working memory in rats. *Behavioural Brain Research, 242*, 84-94.

Sokolowski, J. D., & Salamone, J. D. (1994). Effects of dopamine depletions in the medial prefrontal cortex on DRL performance and motor activity in the rat. *Brain Research, 642*, 20-28.

Spear, L. P. (2000). The adolescent brain and age-related behavioral

manifestations. *Neuroscience and Biobehavioral Reviews, 24*(4), 417-463.

Stewart, P. W., Sargent, D. M., Reihman, J., Gump, B. B., Lonky, E., Darvill, T., et al. (2006).

Response inhibition during differential reinforcement of low rates (DRL) schedules may be

sensitive to low-level polychlorinated biphenyl, methylmercury, and lead exposure in

children. *Environmental Health Perspectives, 114*(12), 192301929.

Thai, C.A., Zhang, Y., Howland, J.G. (2013). Effects of acute restraint stress on set-shifting and

reversal learning in male rats. *Cogn Affect Behav Neurosci*. Mar;13(1):164-73. PubMed

PMID: 23055093.

Weiss, B. (2002). Sexually dimorphic nonreproductive behaviors as indicators of endocrine

disruption. *Environmental Health Perspectives, 110 Suppl 3*, 387-391.

Widholm, J. J., Clarkson, G. B., Strupp, B. J., Crofton, K. M., Seegal, R. F., & Schantz, S. L.

(2001). Spatial reversal learning in aroclor 1254-exposed rats: Sex-specific deficits in

associative ability and inhibitory control. *Toxicology and Applied Pharmacology,* 174:188-

198

Chapter 4: Adolescent Exposure to Polychlorinated Biphenyls, Behavior and Cognitive Functioning.

Abstract

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that are no longer used in industry but still exist in our environment due to their resistance to degradation. Converging human and animal data suggest that perinatal exposure to PCBs is associated with cognitive and behavioral deficits similar to those seen in attention deficit hyperactivity disorder (ADHD), including deficits in executive functions such as cognitive flexibility and response inhibition. Little is known about PCB exposure during another critical period of brain development: adolescence. During adolescence, the prefrontal cortex as well as executive functions and behaviors modulated by this area are maturing. To explore the potential impact of PCB exposure during adolescence on executive functions, data were collected from 115 12-18 year old children of sport anglers in Green Bay, Wisconsin, who were exposed to PCBs through consumption of fish from contaminated waters. PCB concentrations were measured in serum by capillary column gas chromatography with electron capture detection (ECD). Cognitive flexibility was assessed using the CANTAB Intradimensional/Extradimensional (ID/ED) set-shifting task, and response inhibition was tested using the Integrated Visual and Auditory (IVA) Continuous Performance Task. Behaviors associated with ADHD were assessed using the Conner-Wells' Behavior Rating Scale—Parent Report. PCB exposure was not associated with scores on any of the four Conners' scales. In contrast, on the ID/ED set shifting task there was a sex specific association with PCB exposure. Males, but not females, with higher PCB exposure took a greater number of total trials to complete all phases of the task. On the IVA, PCB exposure was not associated with response inhibition in either sex. The results of this study

suggest a subtle deficit in cognitive flexibility associated with PCB exposure in adolescent boys. In contrast, exposure was not associated with a deficit in response inhibition in either girls or boys.

Introduction

        Polychlorinated biphenyls (PCBs) are chemicals that were once used as dielectric fluids in capacitors and transformers as well as in carbonless copy paper, fluorescent light ballasts and caulking material (Ross, 2004). PCBs are also inadvertently produced as a byproduct of the manufacture of paint pigments and thus continue to be found in commercially available products that ultimately terminate in landfills and adjacent ecosystems (Grossman, 2013). Furthermore, older buildings still containing PCBs in caulking and fluorescent light ballasts will continue to contribute PCBs to ecosystems as they are remediated or demolished (Hornbuckle & Robertson, 2010). Despite being banned from production over 4 decades ago, PCBs are still persistent in our environment. In fact, multiple studies have shown that human populations that consume fish from contaminated waters such as the Great Lakes have elevated PCB concentrations in their blood relative to other populations (Schaeffer *et al,* 2006; Turyk *et al,* 2012).

        During the process of de-inking and recycling carbonless copy paper, a number of paper mills in northeastern Wisconsin released large amounts of PCBs into the Fox River. Despite the fact that production of PCBs was banned in the 1970s, PCB levels in the sediment and fish of the Fox River remain elevated to this day, and fishing advisories for the River have been in effect continuously since 1976 (Wisconsin Division of Natural Resources, 2014). Populations still consume fish from these contaminated waters and thus have elevated blood PCB levels. For instance, our group recently reported that Hmong adults fishing in waters of northeastern Wisconsin, including the Fox River, had elevated serum PCB concentrations that were directly correlated with sport-caught fish consumption (Schantz *et al,* 2010).

        Research in humans and animals has demonstrated that PCB exposure during early development is associated with cognitive deficits. In particular, converging evidence from both human and animal studies has suggested that executive functions such as response inhibition and

cognitive flexibility are particularly vulnerable to disruption by PCBs (reviewed in Eubig *et al*, 2010). In a birth cohort of children born to women who consumed PCB-contaminated fish from Lake Michigan, higher umbilical cord serum PCB levels were dose-dependently associated with poorer performance on Fagan's test of visual recognition memory at 7 months of age and poorer verbal and quantitative short-term memory function at 4 years of age (Jacobson *et al,* 1985; Jacobson *et al,* 1990). Prenatal PCB exposure in this cohort was also associated with poorer concentration, greater impulsivity, and poorer verbal, pictorial, and auditory working memory (Jacobson & Jacobson, 1996) as well as lower full-scale and verbal IQ scores, with the strongest effects related to memory, cognitive flexibility and response inhibition when children were tested at 11 year of age (Jacobson & Jacobson, 2003).

Some of the most compelling results of this research were the correlations between PCB exposure and deficits in response inhibition and cognitive flexibility. To assess response inhibition the researchers used a continuous performance task (CPT) in which a child had to press a button only when a target appeared and inhibit responding if a non-target appeared. In this task, errors of commission are scored when the subject fails to inhibit responding to a non-target (thus, assessing impulsivity/response inhibition). Errors of omission, on the other hand, are scored when the subject fails to press when the target appears (thus, measuring inattention). In this task, the Jacobson study (2003) found that 11 year olds with higher prenatal PCB exposure made more errors of commission on the CPT, suggesting a deficit in response inhibition.

The Wisconsin Card Sort Task (WCST) is a task commonly used to assess cognitive flexibility. Here, the child had to sort cards based on 3 dimensions (color, shape, number), and the ability to shift mental set, or set-shift, was assessed by changing the sorting criterion and measuring the number of errors the child made before accurately shifting responses to the correct

dimension. Along with the deficit in response inhibition, children in the Jacobson (2003) study with higher PCB levels also made more errors prior to reaching criterion performance after a set-shift on the WCST, suggesting a deficit in cognitive flexibility.

An Oswego, New York birth cohort study of children born to women who consumed PCB-contaminated Lake Ontario fish found similar results. Prenatal PCB exposure, as measured in umbilical cord serum, was associated with poorer performance on the Fagan test of infant intelligence at 6 and 12 months of age, suggesting a deficit in overall cognitive ability (Darvill *et al,* 2000). Stewart and colleagues also performed a series of cognitive tests using domain-specific tasks of executive function on children of this cohort across different time points. When tested at 4.5 years of age, they found a dose-dependent association between cord serum PCB levels and errors of commission on a CPT in these children (Stewart *et al,* 2003). A similar association was seen between prenatal PCB exposure and increased errors of commission when the children of this cohort were tested again at 8 and 9.5 years of age (Stewart *et al,* 2005). Furthermore, on another task of response inhibition (Differential Reinforcement of Low Rates of Responding; DRL) they found an association between cord serum PCB levels and excessive (impulsive) responding and fewer reinforcers earned in these children at 9.5 years of age (Stewart *et al,* 2006). Taken together, these results provide evidence that prenatal PCB exposure is associated with poorer performance on tasks engaging response inhibition, and this association remains present when children were tested at later time points during childhood.

Although the cohorts described here were exposed to PCBs primarily through fish consumption, a number of other studies have shown similar cognitive deficits in children associated with pre- or perinatal PCB exposure from sources other than fish consumption (Boucher *et al,* 2009; Eubig *et al,* 2010). Furthermore, because PCB related deficits in response

inhibition, working memory and cognitive flexibility share many commonalities with the behavioral deficits seen in attention deficit hyperactivity disorder (ADHD) (Aguiar et al., 2010) behavioral rating scales have been employed in epidemiological studies to assess such aspects of behavior. For example, the Conners' Rating Scale for teachers was used in a cohort of children aged 7-11 years to investigate the associate between prenatal PCB exposure and behaviors associated with ADHD (Sagiv *et al,* 2010). The researchers found a higher risk for ADHD-like behaviors associated with higher levels of prenatal PCB exposure.

Interestingly, rats and monkeys perinatally exposed to PCBs show a similar pattern of deficits on DRL tasks and tasks of cognitive flexibility (set-shifting), indicating a convergence of evidence across human and animal studies of pre- or perinatal PCB exposure (reviewed in Sable & Schantz, 2006 and Eubig *et al,* 2010). All in all, these data suggest that PCB exposure during this critical period of early brain development may be related to adverse cognitive and behavioral outcomes lasting far beyond the exposure period.

Despite the wealth of studies focusing on cognitive deficits related to PCB exposure during the prenatal and early postnatal period, and the evidence that executive functions are affected, very few studies have looked at the impact of PCB exposure during another critical period of brain development: adolescence. Adolescence represents the transition between childhood and adulthood and is broadly defined as the period between 12 to 18 years of age in humans (Brenhouse & Andersen, 2011). This period is characterized by certain hallmarks of behavior across species, including an increase in exploration, novelty seeking and risk taking as well as the refinement of many executive processes (reviewed in Crews & Hodge, 2007). Although executive functioning is present in early development, it undergoes marked refinement and maturation through childhood and especially during adolescence (Anderson *et al,* 2001;

Luna, 2009). In fact, studies done in humans have shown clear age-related improvements in executive function during adolescence. One study in children aged 9 to 18 years found that increasing age from preadolescence (ages 9-12) to early adolescence (ages 13-15) to late adolescence (ages 16-19) was significantly associated with better performance on measures of cognitive set-shifting and response inhibition (Rosso *et al*, 2004). Another study found age-related improvements in performance on a stop signal task of response inhibition with adolescents (age 13-17) performing markedly better than children (age 6-12) (Bedard *et al,* 2002). Another study found that adolescents (age 13-17) made more correct inhibitory responses than children (age 8-12) on a response inhibition task, but this performance did not reach that of adults (age 18-27) (Luna *et al,* 2010). These results illustrate that executive function is continuing to mature through late adolescence.

Given the research on the maturation of executive function throughout adolescence, many studies have been done to explore changes in brain function that could underlie these developmental changes in executive control. Research has suggested that an integration of brain function throughout adolescence underlies the maturation of executive functions, namely continued myelination during this period contributes to a growing ability of the prefrontal cortex to influence the rest of the brain (Olesen *et al,* 2003; reviewed in Luna, 2009). One method that has been used to investigate functional circuits involved in cognitive processing across the lifespan is neuroimaging. Functional MRI (fMRI) studies that have looked at performance on tasks of response inhibition have found poorer performance in childhood that steadily improves with age, reaching adult levels of performance in mid to late adolescence (Luna *et al,* 2010). These studies also suggest that functional connectivity of the frontal regions to other areas of the brain is associated with these improvements in inhibitory control. For instance, a study done by

Stevens *et al* (2007) examined functional connectivity during a go/no-go task of response inhibition and found that changes in fronto-striatal-thalamic network activity from adolescence (age 11-17) to adulthood (age 18-37) were related to changes in performance across age. Furthermore, fMRI studies done by Huttenlocher (1990) found that the prefrontal cortex – a region of the frontal lobe of the brain thought to be crucial in proper executive functioning - undergoes late structural changes extending into mid-adolescence (around 16 years of age). All in all, these studies suggest that because the brain area critical to proper executive functioning is maturing during this period, adolescence may be a particularly sensitive period for PCB-related cognitive effects.

In one of the few published studies that has assessed the relationship between current PCB concentrations and cognitive function during adolescence, Newman *et al* (2006) found that serum PCB concentrations were associated with impaired performance on two measures of long-term memory and a measure of reasoning ability in adolescent subjects of the Akwesasne Mohawk Nation. This cohort consisted of adolescents aged 10-17 exposed both prenatally and concurrently to PCBs through consumption of contaminated fish and wild game. The assessments done in this cohort of adolescents, however, focused primarily on memory functioning, processing speed, intellectual ability, reasoning ability and overall cognitive functioning (Newman *et al,* 2006; Newman *et al,* 2009) and did not directly assess executive functions that are maturing in concert with the prefrontal cortex during adolescence. One other study done in a cohort of adolescents found no significant association between PCB exposure and measures of sustained and selective attention (Vermeir *et al,* 2005); however, this is not surprising as attentional deficits have not generally been reported in association with prenatal exposure either (Eubig *et al,* 2010). Furthermore, this study only measured "dioxin-like" PCBs

which are not believed to contribute significantly to deficits in executive function seen with early PCB exposure (Newman *et al,* 2009; Sable & Schantz, 2006). All in all, the paucity of research on PCB exposure during adolescence highlights the need for additional studies that focus on the impact of exposure during this critical period of brain maturation.

Taken together, the evidence presented above led us to hypothesize that PCB exposure during adolescence would be associated with impairments in executive functioning. In the present study, we assessed neuropsychological function in adolescents 12-18 years of age at high risk for exposure to PCBs through consumption of fish from the Lower Fox River and other contaminated waters in northeastern Wisconsin. We measured blood PCB concentrations and assessed the association of PCB exposure to behavioral ratings on the Conners' Parent Report, as well as performance on the Cambridge Neuropsychological Test Automated Batter (CANTAB) Intradimension/Extradimensional Set-Shifting Task (ID/ED) and the Integrated Visual and Auditory Continuous Performance Task (IVA), tasks assessing cognitive flexibility and response inhibition, respectively.

Methods

From 2007 to 2012, a sample of 12-18 year old children were recruited from households in the Green Bay, Wisconsin area identified through the Wisconsin Department of Natural Resources (WDNR) licensed angler database as having a valid Wisconsin fishing license during the 2006-2007 fishing season. All procedures were approved by the Institutional Review Boards (IRB) of the collaborating Universities, and all participants gave written informed consent.

Wisconsin residents who purchased fishing licenses in 2006-2007 were asked to check a box giving permission for the release of their personal identifying information to outside parties, including biomedical researchers. From the 857,353 individuals in the database who gave this

permission, we established a sampling frame for our study by searching the database for licensed

anglers between 32 and 65 years of age residing in 8 zip codes representing the greater Green

Bay and De Pere metropolitan areas in northeastern Wisconsin. We chose this age range as

individuals in this age range were most likely to have adolescent children in the household. Our

final sample included 20,386 individuals meeting these age criteria. Phone numbers were

obtained for 92% of the sample, or 18,720 individuals. We then matched people by address of

residence, yielding 15,528 unique households with at least one licensed angler 32-65 years of

age. For recruitment purposes, we selected a 10% random sample of angler households from

each zip code and mailed information sheets describing the study in batches of 250. A few days

later, we began trying to contact the homes by telephone to determine if there were eligible

children (age 12-18) in the home and, if so, their willingness to participate. If a family with an

eligible child agreed to participate, an appointment was scheduled for the child and a parent to

visit our research office to complete the assessment battery and questionnaires. The final sample

for the study included 115 children from 102 households.

Neuropsychological Testing

Set-Shifting

Subjects came to our field research office in Green Bay, WI for testing. They were

administered the Intradimensional/Extradimensional (ID/ED) set-shifting task from the

Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB contains a

variety of tests that interrogate a wide range of cognitive domains including memory, attention

and executive function (Fray & Robbins, 1996). The test was administered using a computer

with a touch sensitive screen. The ID/ED set-shifting task took about 15 minutes to complete and

assessed the ability of the subject to attend to a specific dimension of a compound stimulus and

then shift attention to the previously irrelevant dimension when required. The series began with a simple shape discrimination (simple discrimination; SD): 2 shapes were presented, one correct and the other incorrect. Feedback taught the subject which shape was the correct choice. After 6 consecutive correct responses (criterion), the rule changed and the previously incorrect shape became correct (simple reversal; SR). After criterion was met, another reversal was given, but this time irrelevant cues (white lines) were shown next to the shapes (compound discrimination 1; CD1). In the next stage, the white lines were superimposed over the shapes, but shape was still the correct dimension (compound discrimination 2; CD2). After criterion was met, an intradimensional shift (IS) took place whereby new shapes and lines were presented, but shape was still the relevant dimension. After criterion was met, the same shapes and lines from the stage before were presented, but the opposite shape was now correct (intradimensional reversal; IR). After criterion was met, an extradimensional shift (ES) occurred in which the lines became the relevant stimulus dimension. In the final stage, the same shapes and lines from the stage before were presented, but the opposite line was now correct (extradimensional reversal; ER). Subjects progressed through the test by satisfying a set criterion of learning at each stage (6 consecutive correct responses). If at any stage the subject failed to reach this criterion after 50 trials, the test terminated. Data were analyzed as count variables. The dependent variables included trials to criterion at each stage and total trials to complete all stages of the task.

The IVA

The Integrated Visual & Auditory Continuous Performance Test (IVA Plus; Sandford & Turner, 2009) is a computerized continuous performance test (CPT) that combines auditory and visual stimuli to measure attention, impulsivity and hyperactivity. The test presented 5 blocks of 100 trials (totaling 500 trials) of "1"s and "2"s, shifting sets between the visual and auditory

modalities. The subject was required to click the mouse only when he/she saw or heard a 1 (target stimulus) and to inhibit clicking when he/she saw or heard a 2 (non-target stimulus). The presentation frequency of the stimuli were varied during different portions of the test in order to assess both errors of commission (impulsivity) and omission (inattention). Specifically, during the first 50 trials of a 100-trial block, the target stimulus was presented on 42 of the trials and the non-target stimulus on 8 trials. Here, the 1's are more common than the 2's, creating a response set that invites errors of commission. In the second 50 trials of a 100-trial block, the target was presented on 8 of the trials and the non-target on 42 trials. Here, the 1's occur rarely, inviting more errors of omission since the subject must remain vigilant for the target. The presentation of visual and auditory stimuli was equally balanced in each 100-trial block. The IVA lasted approximately 15 minutes. The IVA provides measures of inattention and impulsivity (response inhibition) based on reaction time and errors of omission or commission, respectively. An error of commission occurred when the subject clicked at a non-target (a "2"). Composite scores were calculated for auditory and visual stimuli separately. Composite percent raw scores for the response inhibition measure, based on errors of commission, were calculated as follows: $100 - ((\text{number of auditory errors}/75)*100)$, and $100 - ((\text{number of visual errors}/65)*100)$.

Inattention was assessed by errors of omission. An error of omission occurred when a subject failed to respond to a target (a "1"). Composite scores were calculated for auditory and visual stimuli separately. Composite percent raw scores for the inattention measure, based on errors of omission, were determined as follows: $100 - ((\text{number of auditory errors}/45)*100)$ and $100 - ((\text{number of visual errors}/45)*100)$.

We also assessed sustained effort over time by measuring the mean reaction times (RTs) of correct responses to targets during the first 200 trials compared to those of the last 200 trials.

This measure assesses the difficulty the subject may have in maintaining speed of mental processing throughout the task. Scores were calculated for the auditory and visual modalities separately. Raw percent scores were calculated as follows: ((mean auditory RT of sets 1 + 2)/(mean auditory RT of sets 4 + 5))*100 and ((mean visual RT of sets 1 + 2)/(mean visual RT of sets 4 + 5))*100. Data were analyzed as continuous variables for all three IVA measures.

Conners' Rating Scale-Revised

The Conners' Parent Rating Scale-Revised (short form) (Conners, 2001) was used to assess ADHD and evaluate problem behavior in subjects. Items on the Conners' parent and self-report forms are divided into 4 behavioral scales: oppositional, cognitive problems/inattention, hyperactivity and the Conners' ADHD index. Raw scores were calculated for each of the 4 scales on the parent rating form and were analyzed as count variables.

Covariates

Parent Questionnaires

Information about possible confounders was gathered through interviews and questionnaires administered to subjects and their parents. When a child and family agreed to participate, a fish consumption questionnaire was mailed to the child's parent to obtain historical and detailed information about current (over the previous 12 months) fish consumption. At the neuropsychological assessment, a parent completed an interview designed to obtain detailed information on family demographic characteristics, lifestyle factors (including maternal smoking and alcohol consumption during her pregnancy with the study child), and the child's medical history. Questions about the child's medical history included a broad range of health conditions that can affect neuropsychological testing including visual/hearing impairments, motor

conditions or injuries, head injury and attentional or behavioral diagnoses.

Subject Tests and Questionnaires

Subjects were also given an interview at the time of neuropsychological testing in order to collect more data on potential confounding variables that could influence performance on the outcome measures of interest. Information about the subject's alcohol consumption and smoking, including length of time and frequency with which the subject has been drinking and/or smoking, was obtained. Number of hours since last cigarette was also ascertained. Use of prescription and over the counter medications prior to and on the day of testing was obtained. At the time of blood draw, subjects also completed a brief interview with questions regarding recent fish consumption and from where the fish came. The Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was used to control for general intellectual functioning. Two subtests of the WASI were administered to the child: Vocabulary and Matrix Reasoning. When administered together, these subtests provide an estimate of overall intellectual functioning that is highly correlated with the WASI full scale IQ score (r=0.93-0.94).

Taken together, covariates that were evaluated as potential confounders in this study were categorized into 4 classes of variables: demographic variables, early-life variables, subject lifestyle variables and psychological variables (Table 1). Among the early-life variables, maternal alcohol consumption was measured by number of months drinking alcohol during pregnancy and frequency of consumption. Maternal cigarette smoking was assessed by number of months smoking cigarettes during pregnancy and number of cigarettes smoked per day on average. Parents were also asked whether the subject experienced any head injuries or near drowning experiences as well as whether the subject had any history of epilepsy/seizures, meningitis, encephalitis, other neurological disorders, high fever, asthma, allergies, frequent ear

infections, diabetes and/or exposure to lead or poisons. Among subject lifestyle variables, caffeine consumption was measured by soda, tea and coffee frequency. Cigarette smoking was measured by a yes/no question. Subjects were also asked when they last smoked a cigarette prior to neuropsychological testing. Alcohol consumption was measured by a yes/no question and frequency of consumption if "yes".

Blood PCB Concentrations

A blood sample was collected from each study participant by a trained phlebotomist within two weeks after the neuropsychological assessment was completed. Samples were analyzed for PCBs and other contaminants in the lab of Dr. Paul Kostyniak at the State University of New York (Buffalo, NY) using a modification of the method described by Greizerstein *et al* (1997). PCB congeners were extracted from the serum using pressurized liquid (n-hexane) extraction. The serum sample was mixed with 4 g Hydromatix and then loaded into 34 ml extraction cells containing 5 g of Florisil. The resulting mixture was then extracted with hexane using Accelerated Solven Extraction (Dionex; Sunnyvale, CA). The extract was evaporated using a Zymark TurbVap II Concentrator (Hopkinton, MA) to a volume of 1 ml. Further cleanup of the extract was performed by eluting through a 500 mg Florisil Sep-Pak (Waters Corp; Milford, MA) solid phase extraction (SPE) cartridge. The extract was evaporated to a final volume of 0.2 ml, and PCB congeners 30 and 204 were added as internal standards. The extract (1 ul) was then injected into an Agilent (Palo Alto, CA) 6890 N Gas Chromatograph, and individual PCB congeners were separated on a 60m x 0.25mm SPB-5 (Supelco, Inc.) fused silica capillary column with electron capture detection.

The identification and quantitation of PCB congeners was accomplished by comparing the sample chromatogram peak areas and relative retention times to those of the PCB congener

calibration standards. Retention times were calculated relative to the internal standards (PCB 30 and PCB 204). If the area for any congener exceeded the initial calibration range, the sample extract was diluted by the factor necessary to bring the response within the calibration range. Results were corrected (blank-subtracted) for the mean concentration of each congener determined in sheep serum blank samples containing very low background levels of PCBs. Results were also adjusted for the percent recovery of the surrogates (PCB 46 and PCB 142), which were added to each sample at the start of the analysis. Calibration curves (second order polynomial) were generated following analysis of AccuStandard PCB congener calibration mix standards (CS-01 and CS-05). The quality control program consisted of analysis of serum samples in batches of six to ten samples with the addition of four quality control samples. The quality control samples consisted of one reagent blank, one matrix (serum) blank, three quality control check samples and one randomly selected duplicate sample. The lowest calibration standard was at a concentration near, but above, the method limit of detection (LOD), and the other concentrations corresponded to the expected range of concentrations found in real samples. The LOD for each PCB congener was dependent on the level of interfering substances in the sample and laboratory background levels rather than instrumental limitations. The LOD of each congener was defined as the average background noise + 3 standard deviations for the given congener determined in serum blank samples. The LOD was typically in the low ng/g range (i.e., 0.01 ng/g). Non-detectable values were given a value of 0, and the values for individual congeners were summed to attain a total PCB concentration for the serum sample.

Total PCBs in serum were normalized for lipid content in sample matrices. In serum, total lipids (TL) were determined from total cholesterol (TC) and trigylcerides (TG) using the equation: $TL = 2.27 \, (TC) + (TG) + 0.623$. TC and TG measurements were made on serum by

adaptation of the enzymatic colorimetric methods used in Wako lipid diagnostic kits to the semiautoanalyzer Cobas Fara II. Lipid calibration standards were used for calibration and quantitation. Two quality control samples (Wako) containing lipids spanning the range normally found in human serum for each analyte were used for each assay.

Total PCBs (ng/g) in each sample were calculated by summing individual congener concentrations detected in the serum sample. Lipid-adjusted PCB values (ng/g) were obtained by dividing the total PCB concentration by the concentration of lipid in the serum. PCB concentrations in serum followed a log-normal distribution. Data were analyzed and reported here with both lipid adjusted and unadjusted values, as the use of lipid adjusted values only has recently been under review (Schisterman *et al*, 2005).

Statistical Analyses

SAS software was used (SAS Institute Inc., Cary, NC) to perform all statistical analyses. Regression analyses were used to assess the relationship between performance on each outcome measure and total PCBs in serum (lipid adjusted and unadjusted). Because the distribution of PCB concentrations in our population was skewed, values of the exposure variable were log-transformed for use in statistical analyses. Data were first analyzed using both adjusted and unadjusted PCB values, and the results did not differ. Here, we report analyses using lipid unadjusted total PCBs only.

Potential confounders were simultaneously controlled for in our analyses. A regression model was developed for each dependent variable that included all individual covariates identified through univariate analyses as being associated with the dependent variable. Critical covariates identified in univariate analyses were age, sex and the WAIS-R IQ score. A sex by exposure interaction term was also included in the analyses to explore whether PCB exposure

impacted males and females differently. Family income and parental education were associated with some of the outcome variables (e.g., score on Conners' hyperactivity scale, trials to criterion on some ID/ED stages, errors of commission in auditory modality of IVA), but when included in the models did not contribute significantly and, thus, these variables were not included in the final models. None of the other covariates were significantly associated with the outcome variables.

Multiple linear regression was used for analysis of normally distributed continuous outcomes, including scores on the IVA measures of response inhibition, attention and sustained effort. For continuous variables, normal checks for outliers and influential observations were based on leverage statistics and standardized residuals (Cook & Weisberg, 1982). Collinearity was assessed by variance inflation factors and condition numbers (Cook & Weisberg, 1982). Negative binomial regression was used for counts variables, including scores on the Conners' scales and total trials on the ID/ED task. Outcomes such as trials to criterion at each ID/ED stage with markedly skewed distributions were dichotomized and analyzed by logistic regression.

Conners' Parent Scale

Raw scores on each of the 4 Conners' Parent Scales (oppositional, cognitive/inattention, hyperactivity, and ADHD index) were treated as count data and analyzed using a negative binomial regression model. The final multivariate model contained age, sex, the WAIS-R IQ score, PCB concentration and the PCB by sex interaction.

ID/ED Shift Task

Trials to criterion at each stage of the ID/ED task were treated as count data. The majority of subjects completed each stage of the task within the first 10 trials while only a smaller subset took a larger number of trials to reach criterion. Due to this skewed distribution,

data for each stage were dichotomized at the lowest number of trials to complete that stage. The data were then analyzed by logistic regression. The total number of trials across all stages of the task was treated as a count variable and analyzed using a negative binomial regression. Age, sex, the WAIS-R IQ score, PCB concentration and the PCB by sex interaction were included in the multivariate models.

IVA CPT Task

The three continuous outcome measures for the IVA CPT (inattention, response inhibition and sustained effort) were analyzed using multiple linear regression. The final multivariate model contained age, sex, the WAIS-R IQ score, PCB concentration and the PCB by sex interaction.

Results

Sample Characteristics and Serum PCB Concentrations

The demographic characteristics of the sample are summarized in Tables 2 and 3. The children ranged in age from 12-18 years of age, and 76% of the children had consumed local sport-caught fish for an average of 11 years (Table 2). Nearly 100% of the children were white, and the households were predominantly middle class with over 70% having total household incomes above $60,000 (Table 3). Only 6% had a household income less than $40,000. Most households were two-income with 94% of fathers and 82% of mothers employed. Over 60% of mothers and fathers earned at least an Associate's degree. Very few of the women reported consuming alcohol or smoking during their pregnancy with the child in the study.

Table 2 shows the total PCB (ng/g lipid) in serum samples collected from the children. Children in this cohort had a mean serum total PCB concentration of 0.2 ng/g (lipid unadjusted) and a lipid adjusted mean concentration of 45.51 ng/g of serum lipid.

Conners' Parent Rating Scale Scores

Estimates of effects analyzed by negative binomial regression for the Conners' scales are listed in Table 4. PCB concentration was not significantly associated with scores on these four scales. IQ had a significant negative association with the raw scores of all four Conners' Scales: oppositional (p=0.0156), cognitive/inattention (p=0.0004), hyperactivity (p=0.0096), and the ADHD index (p=0.0002). In other words, lower IQ scores are associated with higher (worse) scores on each of the Conners' scales. The PCB by sex interaction was not significant for any of the Conners' scales.

ID/ED Shift Task Performance

Negative binomial regression for total trials to complete all nine stages of the task revealed a significant positive association of PCB exposure with total trials in males (p=0.0566; see Figure 10). In females, PCB exposure was not associated with total trials, but in males a 25% increase in PCB concentration was associated with a 6% increase in total trials. As expected, IQ was negatively associated with total trials on this measure (p=0.0076) with higher IQ being associated with fewer total trials to complete the ID/ED task. The results of logistic regressions for trials to criterion at each individual stage are represented as odds ratio estimates (Figure 11). PCB exposure was not significantly associated with trials to criterion on any of the individual stages of the ID/ED task. There was no significant interaction of PCB exposure by sex on trials to criterion at any of the individual stages.   *IVA CPT Performance*

Table 5 shows parameter estimates for two measures on the IVA CPT task: response inhibition and sustained effort. Scores for errors of omission, the measure of inattention in this task, did not show much variation, with many subjects scoring 100%. Thus, inattention scores were not statistically analyzed. PCB concentration was not associated with scores on the response inhibition measure in the auditory or visual modality. Age (p=0.0101), IQ (p=0.0087),

and sex (female) (p=0.0016) were positively associated with performance on this measure as percent raw scores were higher in females and increased with increasing age or increasing IQ. Multiple linear regression revealed a significant positive association between PCB concentration and sustained effort scores in the auditory modality (p=0.0285). In other words, higher PCB concentrations were associated with faster RTs for correct responses over the course of the task. PCB concentration was not significantly associated with sustained effort in the visual modality. The PCB by sex interaction was not significant for any outcome measures assessed in the IVA.

Discussion

In this study, higher PCB exposure was not associated with worse scores on the Conners' behavioral rating scale or poorer response inhibition as measured on the IVA continuous performance task, but there was a sex-specific association between higher PCB exposure and total trials to criterion on the set-shifting task. Boys with higher PCB exposure took a greater number of trials to complete the task.

PCB Exposure

The PCB concentrations in these adolescent fish consumers (0.2 ng/g lipid unadjusted; 45.51 ng/g lipid adjusted) were significantly lower than those that have been reported in adult fish eating populations (Schantz *et al,* 2010), but this was expected given that these compounds are lipophilic and bioaccumulate gradually over the lifespan. Sparse data are available documenting adolescent PCB exposure. A cohort from the Akwesasne Mohawk nation is one of the only other groups of adolescents to date in which PCB exposure has been assessed. The levels observed in the children in our cohort are similar to those reported in the Akwesasne adolescents (mean = 0.7 ng/g; N=271) (Newman *et al*, 2006). Similar to the results of our study,

Newman *et al* (2006) also reported significant, but subtle, associations of adolescent PCB exposure with measures of cognitive function.

ID/ED Shift Task

In this study, adolescent males with higher PCB concentrations took more trials to complete the ID/ED task, but there were no significant associations of PCB exposure with trials to criterion on any of the individual stages of the task. One explanation for this finding may be that, while the trend was for PCB-exposed boys to take more trials to reach criterion at most of the stages (see Figure 10), the task may have not been challenging enough to detect statistically significant decrements in performance related to exposure at any individual stage. It was only when the trials were summed across all stages that this significant association between higher PCB concentrations and poorer performance was detected. Given that PCB exposure was related to an increase in total trials to complete the task, indicating a subtle overall decrement in performance, it is possible that adolescent PCB exposure may adversely impact cognitive flexibility to a greater extent than was obvious in this study. However, a more challenging task may be needed in order to reveal deficits at specific stages of the task such as the ED shift or reversals.

The effect of PCB exposure on cognitive flexibility was seen only in boys in our study. As PCBs are known endocrine disrupters (Crinnion, 2011), differential effects in males and females are not surprising. Most human studies in PCB-exposed cohorts have not described whether sex-specific effects on cognition were seen, but some studies have reported PCB-related effects on sexually dimorphic behaviors. For instance, one study reported that PCBs may modify sex differences in toy preference and play activities in perinatally-exposed children (Winneke *et al,* 2014). A study in a Dutch cohort reported similar findings in regards to play behavior in

school-aged children exposed perinatally to PCBs (Vreugdenhil *et al,* 2002). In this study, they found that higher PCB levels were associated with less masculinized play in boys and more masculinized play behavior in girls. In addition, some animal studies have reported sex-specific effects in rats exposed to PCBs. In fact, the animal study described in chapter 3 found an effect of adolescent-PCB exposure only in males on a set-shifting task engaging cognitive flexibility. Another study in rodents found that perinatal PCB exposure was associated with deficits in performance of a spatial reversal learning task, with exposed males and females showing different patterns of deficits (Widholm *et al,* 2001). Taken together, these studies, including our own, provide evidence that PCB-exposure may have differential effects on various aspects of behavior in males and females.

IVA CPT

Surprisingly, we found that higher PCB exposure was associated with better scores on the sustained effort measure from the IVA CPT, which assessed how well the subject was able to maintain speed of mental processing across time. Specifically, subjects with higher PCB exposure became quicker at making correct responses by the end of the IVA task; however, the association was present only in the auditory modality and the significance of this is unclear.

Contrary to our hypothesis, we did not see PCB-related detriments in performance on the response inhibition measure of the IVA CPT. This was surprising as previous studies assessing prenatal exposure have consistently found an association between higher PCB concentrations and poorer performance on tasks of response inhibition, including CPTs (reviewed in Eubig *et al,* 2010). Interestingly, CPTs have recently been criticized due to their insensitivity and inconsistency in detecting behavioral features of ADHD, especially in adolescents (Berger & Cassuto, 2014). If the IVA CPT does in fact lack sensitivity to consistently discriminate

sustained attention and response inhibition differences in adolescents with ADHD, it is unlikely that this task would detect potentially more subtle differences in performance due to PCB exposure. Furthermore, the fixed inter-stimulus interval in the IVA CPT may have made it easy for adolescents to reach ceiling performance once they figured out the timing of stimulus appearance. Thus, although the IVA CPT may be useful for detecting differences in performance in children, it may not sufficiently engage cognitive demands in adolescents such that subtle deficits in attention or response inhibition may be detected. Future studies should employ CPTs that have been designed specifically for adolescents and adults to be more cognitively taxing and thus more likely to draw out even subtle deficits in response inhibition (Berger & Cassuto, 2014).

Conners' Behavioral Rating Scale

With this scale, we sought to assess whether adolescent PCB exposure would be predictive of behavioral features associated with ADHD. We found that higher PCB concentrations were not associated with higher (worse) scores on the four scales of the Conners' Parent Rating Form, which assess ADHD-related problem behavior in subjects. Newman *et al* (2014) reported a similar lack of association of PCB exposure with Conner's scores in their adolescent cohort. These results are not surprising as previous research has indicated genetic as well as prenatal and early postnatal environmental influences as being risk factors for ADHD (reviewed in Yolton *et al*, 2014), whereas there is little evidence that adolescent exposures are risk factors. Together, the results from the Newman et al (2014) study and the current study suggest that PCB exposure during adolescence may not be predictive of ADHD-like behavior problems.

Conclusions

In this study we reported a sex-specific association of PCB exposure with trials to criterion on a computerized set-shifting task where higher PCB concentrations were related to a greater number of total trials to complete the ID/ED task in males, but we saw no effect of exposure on response inhibition on the IVA CPT. Although the findings of this study were subtle, there was a deficit in performance on the cognitive flexibility task associated with adolescent PCB exposure which suggests there may be vulnerability to PCBs during this period. However, because our subjects were children of sports anglers, it is important to note that serum PCB concentrations in a number of our subjects may reflect PCB exposure throughout the lifespan and not just exposure limited to adolescence. Thus, it is possible that early developmental exposure to PCBs may confound our results, making it difficult to associate these cognitive outcomes with PCB exposure that is limited to the adolescent period only. Another limitation of this study was the small sample size, which limited our power to detect relationships between PCB exposure and cognitive outcome measures. By comparison, the study in Awkesasne Mohawk adolescents had about double the sample size of our study (n=271) (Newman *et al,* 2006). Because there are very few studies assessing the effects of PCB exposure in adolescents to date, and subtle PCB-related deficits have been found in our cohort as well as the Awkesasne cohort, it is necessary to further build on these studies using larger samples and more challenging cognitive tests. Furthermore, it is clear that PCBs will continue to persist in our environment as a result of various sources of exposure new and old (Grossman, 2013). This highlights the need for continued research on the effects of these environmental contaminants on the maturing, plastic, adolescent brain.

Figures and Tables

Table 1: Summary of Covariates

I.	Demographic Variables Subjects' age, sex, parental income, maternal education, paternal education
II.	Early-Life Variables Maternal alcohol consumption, maternal smoking, birth complications, subject injury, subject health problems
III.	Subject Lifestyle Variables Medications, attention problems, caffeine consumption, alcohol consumption, smoking, dominant hand, hours spent reading
IV.	Psychological Variables Overall intellectual function (WAIS-R IQ)

Table 2: Characteristics of subject (n=115)*

Characteristic	N(%)	Mean	Median	Range
Sex (Male)	62(54)			
Age (years)		15.6	16.16	12.63-18.97
Total PCBs (ng/g) - lipid unadjusted		0.2	0.15	0.01-1.86
Total PCBs (ng/g) - lipid adjusted		45.51	33	2.56-389.10
Years consuming sports-caught fish Males Females		 10.7 11.3	 11.5 12.0	 2-18 3-18
Years consuming sports-caught fish (in overall study population)				
0	27(23.48)			
1-5	9(7.83)			
6-11	40(34.78)			
12-18	35(30.43)			
Missing	4(3.48)			

* Reported values are for the sample of 115 adolescent participants who provided a blood sample.

Table 3: Demographic Characteristics of Households*

Characteristic	Mother N (%)	Father N (%)
Race (White)	100 (99)	101 (98)
Education (Associates or higher)	79 (68.1)	75 (63.79)

Mean age at child's birth (years)	29.40	31.52	
Employment status (employed)	84 (82.4)	96 (94.1)	
Household income (>$60,000)	86 (74.14)		

*Reported values are for the sample of 102 households that
had 1-3 adolescent participants (12 out of the 102 households
had more than 1 child participating in the study);
(n=113 for mothers; n=115 for fathers).

Table 4: Parameter estimates for total PCBs on the Conners' behavioral scales*

Conners' Scale Measure	Estimate	Standard Error	p-value
Oppositional	0.135	0.173	0.437
Cognitive Problems/Inattention	-0.072	0.202	0.721
Hyperactivity	0.047	0.217	0.829
ADHD Index	-0.043	0.129	0.741

*(n=115)

Table 5: Parameter estimate for total PCBs on IVA measures*

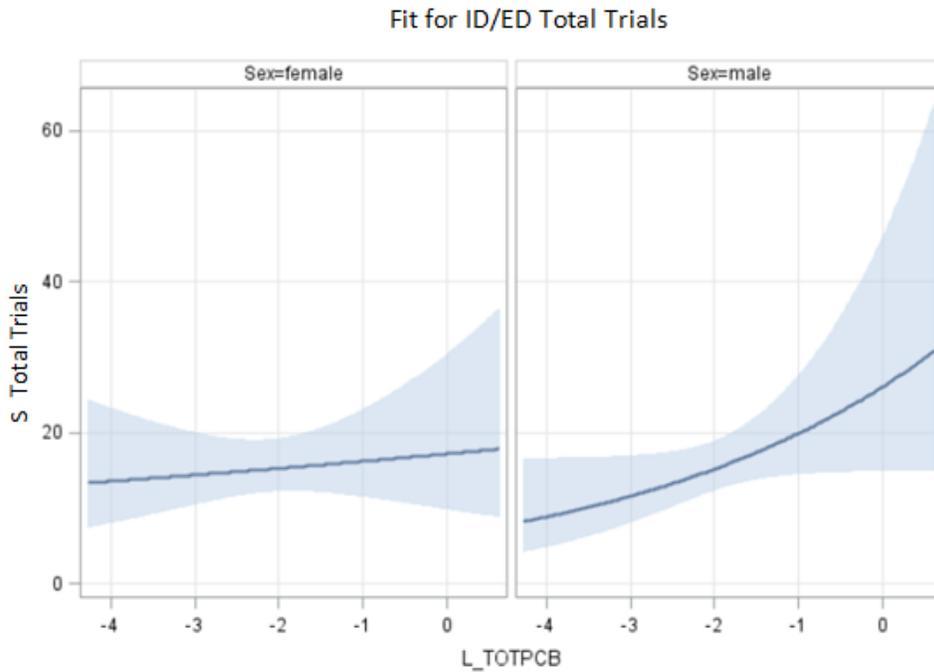| Measure	Estimate	Standard Error	t-value	p-value
Response Inhibition				
Auditory	-0.367	0.468	-0.790	0.434
Visual	0.096	0.732	0.130	0.896
Sustained Effort				
Auditory	2.620	1.179	2.220	0.029
Visual	0.935	1.020	0.920	0.361

*(n=105)

Figure 10: Plot of relationship between PCB concentration and total trials in the ID/ED task computed at mean age=16.07 years and mean IQ=106.7 (with 95% confidence limits). Negative binomial regression for total trials across all nine stages of the task revealed a significant positive association between PCB concentration and total trials in males (p=0.036) (n=111).
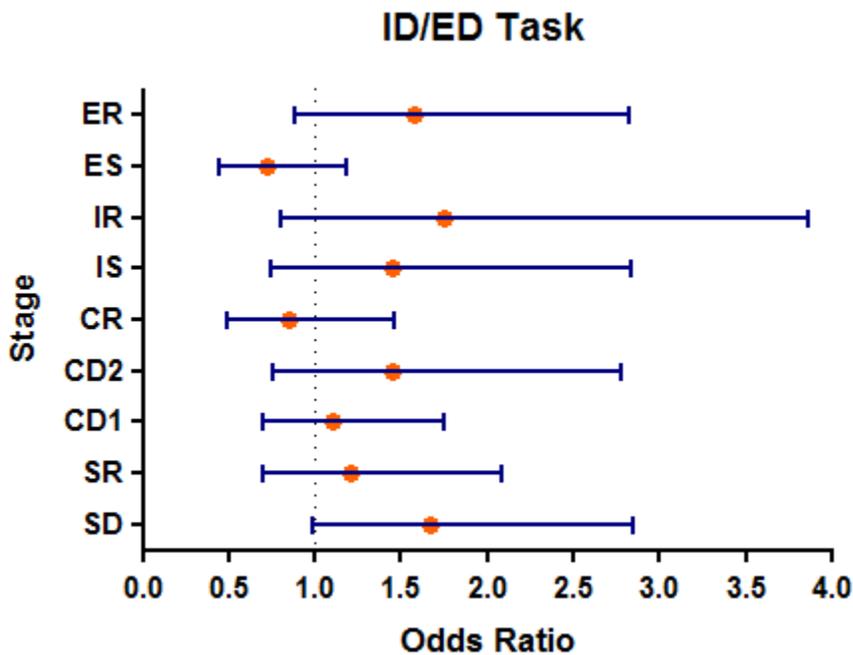

Figure 11: Odds ratios estimates (mean and 95% confidence intervals) for trials to criterion at each ID/ED stage. PCB exposure was not associated with trials to criterion on the ID/ED task at

any individual stage (n=103-111). Abbreviations: Simple Discrimination (SD); Simple Reversal (SR); Compound Discrimination 1 (CD1); Compound Discrimination 2 (CD2); Compound Reversal (CR); Intradimensional Shift (IS); Intradimensional Reversal (IR); Extradimensional Shift (ES); Extradimensional Reversal (ER).

References

Aguiar, A., Eubig, P.A., & Schantz, S.L. (2010). Attention deficit/hyperactivity disorder: a
focused overview for children's environmental health researchers. *Environ Health
Perspect*. 118(12):1646-53.

Anderson, V. A., Anderson, P., Northam, E., Jacobs, R., & Catroppa, C. (2001). Development of
executive functions through late childhood and adolescence in an australian
sample.*Developmental Neuropsychology, 20*(1), 385-406.

Bedard, A. C., Nichols, S., Barbosa, J. A., Schachar, R., Logan, G. D., & Tannock, R. (2002).
The development of selective inhibitory control across the life span. *Developmental
Neuropsychology,21*(1), 93-111.

Berger, I. & Cassuto, H. (2014). The effect of environmental distractors incorporation into a
CPT on sustained attention and ADHD diagnosis among adolescents. *J Neurosci
Methods*. 222:62-8.

Boucher, O., Muckle, G., & Bastien, C. H. (2009). Prenatal exposure to polychlorinated
biphenyls: A neuropsychologic analysis. *Environmental Health Perspectives, 117*(1), 7-16.

Brenhouse, H.C. & Andersen, S.L. (2011). Developmental trajectories during adolescence in
males and females: a cross-species understanding of underlying brain changes. *Neurosci
Biobehav Rev*. 35(8):1687-703.

Brodsky, K., Willcutt, E. G., Davalos, D. B., & Ross, R. G. (2014). Neuropsychological
functioning in childhood-onset psychosis and attention-deficit/hyperactivity

disorder. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 55*(7), 811-818.

Crinnion, W. J. (2011). Polychlorinated biphenyls: Persistent pollutants with immunological, neurological, and endocrinological consequences. *Alternative Medicine Review : A Journal of Clinical Therapeutic, 16*(1), 5-13.

Conners, C. K., Wells, K. C., Parker, J. D., Sitarenios, G., Diamond, J. M., & Powell, J. W. (1997). A new self-report scale for assessment of adolescent psychopathology: Factor structure, reliability, validity, and diagnostic sensitivity. *Journal of Abnormal Child Psychology, 25*(6), 487-497.

Cook, R.D., & Weisberg, S. (1982). *Residuals and Influence in Regression.* Chapman and Hall, New York, NY.

Crews F, He J, Hodge C. (2007). Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav*. 86(2):189-99. Review.

Darvill, T., Lonky, E., Reihman, J., Stewart, P., & Pagano, J. (2000). Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. *Neurotoxicology, 21*(6), 1029-1038.

Eubig, P. A., Aguiar, A., & Schantz, S. L. (2010). Lead and PCBs as risk factors for attention Deficit/Hyperactivity disorder. *Environmental Health Perspectives, 118*(12), 1654-1667.

Floresco, S. B., Magyar, O., Ghods-Sharifi, S., Vexelman, C., & Tse, M. T. (2006). Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting.

Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology,31(2), 297-309.

Fray, P. J., & Robbins, T. W. (1996). CANTAB battery: Proposed utility in neurotoxicology.*Neurotoxicology and Teratology, 18*(4), 499-504.

Greizerstein, H. B., Gigliotti, P., Vena, J., Freudenheim, J., & Kostyniak, P. J. (1997). Standardization of a method for the routine analysis of polychlorinated biphenyl congeners and selected pesticides in human serum and milk. *Journal of Analytical Toxicology, 21*(7), 558-566.

Grossman E. (2013). Nonlegacy PCBs: pigment manufacturing by-products get a second look. *Environ Health Perspect.* 121(3):A86-93.

Hornbuckle K, Robertson L. (2010). Polychlorinated biphenyls (PCBs): sources, exposures, toxicities. *Environ Sci Technol*. 44(8):2749-51.

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex development.*Neuropsychologia, 28*(6), 517-527.

Jacobson, J. L., & Jacobson, S. W. (1996). Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): The michigan and north carolina cohort studies. *Toxicology and Industrial Health,12*(3-4), 435-445.

Jacobson, J. L., & Jacobson, S. W. (1996). Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *The New England Journal of Medicine, 335*(11), 783-789.

Jacobson, J. L., & Jacobson, S. W. (2003). Prenatal exposure to polychlorinated biphenyls and

    attention at school age. *The Journal of Pediatrics, 143*(6), 780-788.

Jacobson, J. L., Jacobson, S. W., & Humphrey, H. E. (1990). Effects of in utero exposure to

    polychlorinated biphenyls and related contaminants on cognitive functioning in young

    children. *The Journal of Pediatrics, 116*(1), 38-45.

Jacobson, S. W., Fein, G. G., Jacobson, J. L., Schwartz, P. M., & Dowler, J. K. (1985). The

    effect of intrauterine PCB exposure on visual recognition memory. *Child

    Development, 56*(4), 853-860.

Luna, B. (2009). Developmental changes in cognitive control through adolescence. *Advances in

    Child Development and Behavior, 37*, 233-278.

Luna, B., Padmanabhan, A., & O'Hearn, K. (2010). What has fMRI told us about the

    development of cognitive control through adolescence? *Brain and Cognition, 72*(1), 101-

    113.

Newman, J., Aucompaugh, A. G., Schell, L. M., Denham, M., DeCaprio, A. P., Gallo, M. V., et

    al. (2006). PCBs and cognitive functioning of mohawk adolescents. *Neurotoxicology and

    Teratology, 28*(4), 439-445.

Newman, J., Behforooz, B., Khuzwayo, A. G., Gallo, M. V., Schell, L. M., & Akwesasne Task

    Force on the Environment. (2014). PCBs and ADHD in mohawk

    adolescents. *Neurotoxicology and Teratology, 42*, 25-34.

Newman, J., Gallo, M. V., Schell, L. M., DeCaprio, A. P., Denham, M., Deane, G. D., et al. (2009). Analysis of PCB congeners related to cognitive functioning in adolescents. *Neurotoxicology, 30*(4), 686-696.

Olesen, P. J., Nagy, Z., Westerberg, H., & Klingberg, T. (2003). Combined analysis of DTI and fMRI data reveals a joint maturation of white and grey matter in a fronto-parietal network. *Brain Research.Cognitive Brain Research, 18*(1), 48-57.

Ross, G. (2004). The public health implications of polychlorinated biphenyls (PCBs) in the environment.*Ecotoxicology and Environmental Safety, 59*(3), 275-291.

Rosso, I. M., Young, A. D., Femia, L. A., & Yurgelun-Todd, D. A. (2004). Cognitive and emotional components of frontal lobe functioning in childhood and adolescence. *Annals of the New York Academy of Sciences, 1021*, 355-362.

Sable, H. J. K., & Schantz, S. L. (2006). Executive function following developmental exposure to polychlorinated biphenyls (PCBs): What animal models have told us. In E. D. Levin, & J. J. Buccafusco (Eds.), *Animal models of cognitive impairment* (). Boca Raton (FL): Taylor & Francis Group, LLC.

Sagiv, S. K., Thurston, S. W., Bellinger, D. C., Tolbert, P. E., Altshul, L. M., & Korrick, S. A. (2010). Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. *American Journal of Epidemiology, 171*(5), 593-601.

Sandford, J.A., & Turner, A. (2009). *IVA+Plus Integrated Visual and Auditory Performance Test administration manual.* Richmond, VA: Brain Train.

Schaeffer, D. J., Dellinger, J. A., Needham, L. L., & Hansen, L. G. (2006). Serum PCB profiles in native americans from wisconsin based on region, diet, age, and gender: Implications for epidemiology studies. *The Science of the Total Environment, 357*(1-3), 74-87.

Schantz, S. L., Gardiner, J. C., Aguiar, A., Tang, X., Gasior, D. M., Sweeney, A. M., et al. (2010). Contaminant profiles in southeast asian immigrants consuming fish from polluted waters in northeastern wisconsin. *Environmental Research, 110*(1), 33-39.

Schisterman, E. F., Whitcomb, B. W., Louis, G. M., & Louis, T. A. (2005). Lipid adjustment in the analysis of environmental contaminants and human health risks. *Environmental Health Perspectives, 113*(7), 853-857.

Stevens, M. C., Kiehl, K. A., Pearlson, G. D., & Calhoun, V. D. (2007). Functional neural networks underlying response inhibition in adolescents and adults. *Behavioural Brain Research, 181*(1), 12-22.

Stewart, P. W., Sargent, D. M., Reihman, J., Gump, B. B., Lonky, E., Darvill, T., et al. (2006). Response inhibition during differential reinforcement of low rates (DRL) schedules may be sensitive to low-level polychlorinated biphenyl, methylmercury, and lead exposure in children. *Environmental Health Perspectives, 114*(12), 192301929.

Stewart, P., Fitzgerald, S., Reihman, J., Gump, B., Lonky, E., Darvill, T., et al. (2003). Prenatal

PCB exposure, the corpus callosum, and response inhibition. *Environmental Health*

Perspectives,111(13), 1670-1677.

Stewart, P., Reihman, J., Gump, B., Lonky, E., Darvill, T., & Pagano, J. (2005). Response

inhibition at 8 and 9 1/2 years of age in children prenatally exposed to

PCBs. *Neurotoxicology and Teratology,27*(6), 771-780.

Stewart, P. W., Reihman, J., Lonky, E. I., Darvill, T. J., & Pagano, J. (2003). Cognitive

development in preschool children prenatally exposed to PCBs and MeHg. *Neurotoxicology*

and Teratology, 25(1), 11-22.

Turyk, M. E., Bhavsar, S. P., Bowerman, W., Boysen, E., Clark, M., Diamond, M., et al. (2012).

Risks and benefits of consumption of great lakes fish. *Environmental Health*

Perspectives, 120(1), 11-18.

Vermeir, G., Viaene, M., Staessen, J., Hond, E. D., & Roels, H. A. (2005). Neurobehavioural

investigations in adolescents exposed to environmental pollutants. *Environmental*

Toxicology and Pharmacology, 19(3), 707-713.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence.* The Psychological

Corporation: Harcourt Brace & Company. New York, NY.

Widholm, J. J., Clarkson, G. B., Strupp, B. J., Crofton, K. M., Seegal, R. F., & Schantz, S. L.

(2001). Spatial reversal learning in aroclor 1254-exposed rats: Sex-specific deficits in

associative ability and inhibitory control. *Toxicology and Applied Pharmacology, 174*, 188-198.

Winneke, G., Ranft, U., Wittsiepe, J., Kasper-Sonnenberg, M., Fürst, P., Krämer, U., Seitner, G., & Wilhelm, M. (2014). Behavioral sexual dimorphism in school-age children and early developmental exposure to dioxins and PCBs: a follow-up study of the Duisburg Cohort. *Environ Health Perspect*. 122(3):292-8.

Wisconsin Department of Natural Resources (2014). Choose Wisely: A health guide for eating fish in Wisconsin. www.dnr.wi.gov.

Vreugdenhil, H.J., Slijper, F.M., Mulder, P.G., &Weisglas-Kuperus, N. (2002). Effects of perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age. *Environ Health Perspect*. 110(10):A593-8.

Yolton, K., Cornelius, M., Ornoy, A., McGough, J., Makris, S., & Schantz, S. (2014). Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood. *Neurotoxicol Teratol*. 44:30-45.

Chapter 5: Overall Conclusions

The overarching goal of this thesis was to investigate how PCB exposure during adolescence, a critical period of brain development, would affect cognition using both an animal model and a human epidemiological study. We hoped to generate convergent data in these parallel human and animal studies thus providing stronger evidence that PCB exposure during adolescence is a human health risk than would either approach alone. In the studies presented, we employed tasks engaging cognitive flexibility and response inhibition in both humans and animals exposed to PCBs throughout adolescence.

Both studies found sex-specific effects of PCBs on cognitive flexibility that were present only in males, but the nature of the effect was different in rats and humans. PCB-exposed rats showed an apparent facilitation of learning on the last phase of a set-shifting task, which was a reversal of position. We speculated that this could be due to these rats employing a simpler, more "habit-based" response strategy rather than learning the actual position reversal relevant to the task. Specifically, the original position discrimination required rats to press the lever opposite their side bias. On the position reversal, PCB-exposed animals may have simply reverted to their previous side-bias rather than learning the new response strategy. In human adolescents, on the other hand, we found that boys with higher PCB exposure took significantly more trials to complete all stages of a set-shifting task, but there were no significant differences in trials to reach criterion on any of the individual stages in the series. We speculated that each individual stage of the task may not have been challenging enough to observe significant deficits while, when examined overall, an apparent deficit in performance was revealed.

There were no PCB-related effects on response inhibition in the animal or human study. In the animal study, there were some subtle differences between groups during training on DRL,

but there were no group differences on performance on the DRL15 task itself. Specifically, PCB exposure did not affect total presses or the ratio of reinforced:non-reinforced responses in this task. Similarly, in the human cohort we found no differences in response inhibition, per se. However, we did find, unexpectedly, that PCB exposure was associated with a small but statistically significant improvement in sustained effort in the auditory modality only, as indicated by declining reaction times throughout the course of the task.

In the animal study we looked at DAT expression as an underlying indicator of disrupted DA function that could contribute to differences in performance on the cognitive tasks, but we did not see exposure related differences in the expression of this protein in either the orbital frontal cortex or striatum. Given that the results from our cognitive tasks were subtle and not in the direction we hypothesized, it is not surprising that we did not observe long-term changes in DAT expression in these related brain regions. Taken together, these studies revealed some subtle, sex-specific associations of adolescent PCB exposure with performance on tests of cognitive flexibility that will be interesting to explore further.

PCBs and Executive Functions

Both the human and the animal studies revealed PCB-related changes in cognitive flexibility (i.e., on the set-shifting task) that were present only in males. However, the pattern of effects on set-shifting differed in the human and animal studies. Although there were parallels between the tasks used in the two studies (i.e., shifting from one dimension to another), there were also some differences that could have contributed to the different pattern of effects we observed in rats and humans. In the animal study, rats were required to shift from a visual cue discrimination to a spatial position discrimination and then learn a reversal of spatial position. Because the position reversal involved responding to the position animals were shown to prefer

115

during side-bias testing, it may be that the PCB-exposed animals defaulted to an unextinguished side-bias rather than actually learning a new strategy. If this was the case, the animals were not necessarily engaging cognitive flexibility. To remove this potential for "habit-based" responding, in future studies a dimension other than lever position (e.g., odor) could be used such that rats must learn the new dimension and shift response strategy accordingly in order to earn a reinforcer. In this way, we would be able to more specifically engage strategy shifting and thus cognitive flexibility without confounding from habit-based responding. In the human study by contrast, there was no strategy that could be used other than to learn the new dimension presented at each stage. Thus, a decrement in performance would be reflected in trials to reach criterion at each individual stage or total trials to complete the task. The lack of a significant difference in performance at any of the individual stages suggests that the discrimination at each individual stage may not have been challenging enough to reveal an effect of PCB exposure. However, PCB exposure was still related to an increase in total trials to complete the task suggesting an overall decrement in performance. Thus, the results suggest that adolescent PCB exposure may adversely impact cognitive flexibility, but do not reveal whether specific aspects such as learning an intra-dimensional or extra-dimensional shift or a reversal were affected. A more challenging task may be needed in order to determine whether there are deficits in these specific aspects of cognitive flexibility. In summary, PCB-exposure was related to male-specific differences in performance on set-shifting tasks in both rats and humans, and it would be interesting to explore these differences further using more difficult or multidimensional set-shifting tasks to better understand the nature of the effects.

The sex-specific effect of PCB exposure in these studies is not unexpected as PCBs are known endocrine disruptors (Crinnion, 2011). Most human studies in PCB-exposed cohorts have

not described whether sex-specific effects on cognition were seen, but some studies have reported PCB-related effects on sexually dimorphic behaviors (Winneke *et al,* 2014; Vreugdenhil *et al,* 2002). In addition, some animal studies have reported sex-specific effects in rats exposed to PCBs. For instance, a previous study done in our lab found deficits on DRL15 performance due to perinatal PCB exposure, and this effect seemed to be driven by poorer performance in males (Sable *et al,* 2009). The authors hypothesized that this sex difference may be due to PCB-induced reductions in aromatase activity (Hany *et al,* 1999), an enzyme responsible for the conversion of testosterone to estradiol, which may influence proper sexual differentiation of the brain as well as the proper development of the DA system in the PFC of the developing male rat (Stewart & Rajabi, 1994). Likewise, aromatization of testosterone into estradiol during adolescence seems to play a critical role in the emergence of adult-like male-specific behaviors (Kellogg & Lundin, 1999). Furthermore, testosterone has been implicated in modulating midbrain DA circuits during adolescence (Purves-Tyson *et al,* 2012). Thus, if PCBs are reducing aromatase activity during adolescence, this excess testosterone availability may be differentially influencing DA system function and DA-mediated behaviors in male rats. Data are not as clear in regards to the effects, if any, of PCB on aromatase in humans, although there is sparse evidence that suggests that aromatase may not be active in the male testes prior to puberty (Brodie *et al,* 2001). One study done in Flemish adolescents living in areas with different pollutant exposures (e.g., organochlorines and metals in industrial areas, pesticides in rural areas) reported no significant difference in testosterone or aromatase levels compared to a reference mean in adolescents with the highest PCB exposures, but these results may be confounded by the multitude of other pollutants the subjects were exposed to (Schroijen *et al,* 2008; Croes *et al,* 2009). Although the exact mechanism by which PCBs may be disrupting the endocrine system in

humans may be unknown, differential effects of exposure have been seen in our study as well as in other PCB-exposed cohorts which highlights the importance of further investigation in future studies.

We did not see PCB-related deficits in performance on response inhibition as hypothesized. We speculated that in both studies, subjects were performing the tasks to a degree of efficiency that may have not allowed us to detect any subtle differences in performance. Rats in our study were trained on the set-shifting task prior to DRL training, and we conjectured that the lack of an effect of PCB exposure on response inhibition on the DRL task may have been related to the fact that the rats had this prior experience. To avoid this potential transfer of learning issue, future studies should only test each rat on one task. In the human study, we speculate that the lack of an effect of exposure on the response inhibition measure of the IVA may be because the IVA did not sufficiently engage cognitive demands in adolescents such that subtle deficits on response inhibition could be detected. In fact, continuous performance tasks (CPTs) have recently been criticized due to their insensitivity and inconsistency in detecting behavioral features of ADHD, especially in adolescents (Berger & Cassuto, 2014). If the IVA CPT does in fact lack sensitivity to consistently discriminate response inhibition differences in adolescents with ADHD, it is unlikely that this task would detect potentially more subtle differences in performance due to PCB exposure. Whereas in animals the lack of effect may be due to transfer of learning, in humans the lack of an effect may be due to a CPT that lacked sensitivity. Future studies should use a CPT designed specifically for adolescents and adults that is more cognitively taxing and more likely to be sensitive to subtle deficits in response inhibition.

Aside from maturation of executive functions such as cognitive flexibility and response inhibition during adolescence, another hallmark of this period is increased exploration and

novelty seeking in both humans and animals (reviewed in Crews & Hodge, 2007). In line with this, an important area of research is drug-seeking behavior and the effects of drug exposure during this period (reviewed in Bava & Tapert, 2010). Research in humans and animals has suggested that exposure to drugs such as alcohol or psychostimulants during this period could have cognitive and behavioral consequences lasting into adulthood (reviewed in Gulley & Juraska, 2013). For instance, Sherill *et al* (2013) found amphetamine-induced impairments on a working memory task. Another study found differential effects of amphetamine exposure on cognitive flexibility depending on age of exposure (adolescence versus adulthood) (Hankosky *et al*, 2013). Relevant to this are studies that have explored potential interactions between early PCB exposure and administration of psychostimulants on cognition and behavior and have found differential effects of drug treatment in PCB-exposed vs control animals (Sable *et al*, 2009; Sable *et al*, 2011; Poon et al, 2013). Thus, an important avenue of further research would be to investigate how drugs and chemicals, which adolescents may be exposed to concurrently, could be interacting to alter development of the plastic, maturing brain.

PCB Exposure

The doses of PCBs that we used in our animal study were chosen based on perinatal studies in our lab that found PCB-related effects on response inhibition (Sable *et al,* 2009). Because this study was the first to assess adolescent exposure to PCBs, we explored this paradigm using the same doses that were previously shown in our lab to result in deficits in executive functions after perinatal exposure. The perinatal exposure paradigms used in our lab, however, establish a PCB body burden in dams that is ultimately passed on to the pup *in utero* and lactationally. PCBs that have bioaccumulated in the dam are very effectively transferred to pups via lactation (Lee *et al,* 2002), so the dose that the offspring is receiving may actually be

greater by this route. Due to the shorter exposure period (i.e., adolescence) and less dramatic

brain development occurring during this period in comparison to the perinatal period, future

studies may need to use higher doses to see long-term effects from adolescent exposure.

The adolescents in our human cohort had PCB concentrations somewhat lower than those

of the Akwesasne Mohawk adolescents, one of the only other adolescent cohorts in which PCB

exposure has been studied (Newman *et al,* 2006). Newman and colleagues (2006) also reported

significant, but subtle, associations of PCB exposure with measures of cognitive function. Both

the Awkesasne adolescents and the adolescents in our study come from populations that were

exposed to PCBs primarily through consumption of contaminated fish. As discussed in previous

chapters, the human population is also exposed to airborne PCBs from a variety of sources (e.g.,

landfills, demolition of contaminated buildings, PCBs in caulking material and fluorescent light

ballasts of older buildings including schools). The pattern of PCBs found in air is different from

that found in contaminated fish in that it consists primarily of lightly chlorinated PCBs (Beyer &

Bizuik, 2009), many of which are known to be neurotoxic (Simon *et al,* 2007). Thus, it would be

interesting to study adolescent populations living near PCB-contaminated sites or attending

PCB-contaminated schools to see how airborne PCB exposure in these adolescents may affect

executive function. It would also be interesting to test a PCB mixture representative of that seen

in air samples in animal studies. In fact, one research group has generated PCB vapors from a

"Chicago Air Mixture" that was developed to mimic the PCB congener profile in Chicago air

and is currently using this in inhalation toxicology studies in rats (Zhao *et al,* 2010). Studies

assessing the toxicological effects of inhalation exposure to this mixture have found specific

patterns of sequestration of PCBs in tissue with minimal overt toxic effects (Hu *et al,* 2012);

however, neurotoxic effects may still be present in the absence of any overt toxicity, so more

research is needed to evaluate this air mixture of PCBs. As for cognitive testing in these studies, it would be important to choose tasks that are both sensitive enough to detect even subtle differences in performance and that would yield findings that can be relatable across humans and animals. Then, if compelling cognitive effects are detected with either type of PCB mixture, it would be interesting to do a more thorough assessment of any related changes in the DA system in brain regions relevant to task performance.

Our animal study was the first to look at the long-term effects of adolescent PCB exposure on cognition and neurochemistry, and our human study is one of very few studies that have looked at adolescent PCB exposure and cognition. It is possible, however, that our cohort may have been exposed to PCBs throughout their lifespan, making it difficult to attribute the cognitive effects seen to exposure only during the adolescent period. This highlights the advantage of designing a parallel animal study in which confounding factors inherent in epidemiological studies, such as exposure history, can be carefully controlled for. Thus, together, the parallel human and animal studies presented here provide more compelling evidence - than would either study alone - of PCB-related effects, albeit subtle, on executive functioning.

Unfortunately, it is very clear that PCBs will continue to enter our environment, so continued research on the effects of these chemicals on the human population are necessary. Moreover, because research in adolescent exposure paradigms is still young, especially in neurotoxicology, much more research is needed in this arena in order to generate validated and replicable results. During this period of multidimensional growth, adolescents are exploring their environments more independently as the innumerable environmental influences they are being exposed to are shaping brain, cognition and behavior making research on the effects of

neurotoxicants and other environmental factors a valuable contribution to our understanding of

this critical period of development.

References

Bava, S., & Tapert, S. F. (2010). Adolescent brain development and the risk for alcohol and other drug problems. *Neuropsychology Review, 20*(4), 398-413.

Berger, I. & Cassuto, H. (2014). The effect of environmental distractors incorporation into a CPT on sustained attention and ADHD diagnosis among adolescents. *J Neurosci Methods*. 222:62-8.

Beyer, A. & Biziuk, M. (2009). Environmental fate and global distribution of polychlorinated biphenyls. *Rev Environ Contam Toxicol.* 201:137-58.

Crews, F., He, J., & Hodge, C. (2007). Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav*. 86(2):189-99. Review.

Croes, K., Baeyens, W., Bruckers, L. Den Hond, E., Koppen, G., Nelen, V., Van de Mieroop, E., Keune, H., Dhooge, W., Schoeters, G., & Van Larebeke, N. (2009). Hormone levels and sexual development in Flemish adolescents residing in areas differing in pollution pressure. *Int Journal of Hygiene and Environmental Health.* 212(6):612-25.

Gulley, J. M., & Juraska, J. M. (2013). The effects of abused drugs on adolescent development of corticolimbic circuitry and behavior. *Neuroscience, 249*, 3-20.

Hankosky, E. R., & Gulley, J. M. (2013). Performance on an impulse control task is altered in adult rats exposed to amphetamine during adolescence. *Developmental Psychobiology, 55*(7), 733-744.

Hany, J., Lilienthal, H., Sarasin, A., Roth-Harer A., Fastabend, A., Dunemann, L., Lichtensteiger, W., & Winneke, G. (1999). Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicology and Applied Pharmacology.* 158(3):231-43.

Hu, X., Adamcakova-Dodd, A., Lehmler, H.J., Hu, D., Hornbuckle, K., & Thorne, P.S. (2012). Subchronic inhalation exposure study of an airborne polychlorinated biphenyl mixture resembling the Chicago ambient air congener profile. *Environ Sci Technol.* 46(17):9653-62.

Kellogg, C.K. & Lundin, A. (1999). Brain androgen-inducible aromatase is critical for adolescent organization of environment-specific social interaction in male rats. *Hormones and Behavior.* 35(2):155-62.

Lee, S.K., Ou, Y.C., & Yang, R.S. (2002). Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in nonpregnant mice, lactating mice, and suckling pups. *Toxicol Sci.* 65(1): 26-34.

Newman, J., Aucompaugh, A. G., Schell, L. M., Denham, M., DeCaprio, A. P., Gallo, M. V., et al. (2006). PCBs and cognitive functioning of mohawk adolescents. *Neurotoxicology and Teratology, 28*(4), 439-445.

Poon, E., Monaikul, S., Kostyniak, P. J., Chi, L. H., Schantz, S. L., & Sable, H. J. (2013). Developmental exposure to polychlorinated biphenyls reduces amphetamine behavioral sensitization in long-evans rats. *Neurotoxicology and Teratology, 38*, 6-12.

Purves-Tyson, T.D., Handelsman, D.J., Double, K.L., Owens, S.J., Bustamante, S., & Weickert, C.S. (2012). Testosterone regulation of sex steroid-related mRNAs and dopamine-related mRNAs in adolescent male rat substantia nigra. *BMC Neurosci.* 13:95

Sable, H. J., Eubig, P. A., Powers, B. E., Wang, V. C., & Schantz, S. L. (2009). Developmental exposure to PCBs and/or MeHg: Effects on a differential reinforcement of low rates (DRL) operant task before and after amphetamine drug challenge. *Neurotoxicology and Teratology, 31*(3), 149-158.

Sable, H. J., Monaikul, S., Poon, E., Eubig, P. A., & Schantz, S. L. (2011). Discriminative stimulus effects of cocaine and amphetamine in rats following developmental exposure to polychlorinated biphenyls (PCBs). *Neurotoxicology and Teratology, 33*(2), 255-262.

Schroijen, C., Baeyens, W., Schoeters, G., Den Hond, E., Koppen, G., Bruckers, L., Nelen, V., Van De Mieroop, E., Bilau, M., Covaci, A., Keune, H., Loots, I., Kleinjans, J., Dhooge, W., & Van Larabeke, N. (2008). Internal exposure to pollutants measured in blooc and uring of Flemish adolescents in function of area of residence. *Chemosphere*. 71:1317-25.

Sherrill, L. K., Stanis, J. J., & Gulley, J. M. (2013). Age-dependent effects of repeated amphetamine exposure on working memory in rats. *Behavioural Brain Research, 242*, 84-94.

Simon, T., Britt, J.K., & James, R.C. (2007). Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. *Regul Toxicol Pharmacol*. 48(2):148-70.

Stewart, J. & Rajabi, H. Estradiol derived from testosterone in prenatal life affects the

    development of catecholamine systems in the frontal cortex in the male rat. (1994). *Brain*

    *Res.*646(1):157-60.

Winneke, G., Ranft, U., Wittsiepe, J., Kasper-Sonnenberg, M., Fürst, P., Krämer, U., Seitner, G.,

    & Wilhelm, M. (2014). Behavioral sexual dimorphism in school-age children and early

    developmental exposure to dioxins and PCBs: a follow-up study of the Duisburg Cohort.

    Environ Health Perspect. 122(3):292-8.

Vreugdenhil, H.J., Slijper, F.M., Mulder, P.G., &Weisglas-Kuperus, N. (2002). Effects of

    perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age.

    Environ Health Perspect. 110(10):A593-8.

Zhao, H.X., Adamcakova-Dodd, A., Hu, D., Hornbuckle, K.C., Just, C.L, Robertson, L.W.,

    Thorne, P.S., & Lehmler, H.J. (2010). Development of a synthetic PCB mixture

    resembling the average polychlorinated biphenyl profile in Chicago air. *Environ Int*.

    36(8):819-27.