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FIFTY YEARS OF PCB RESEARCH: NEW APPROACHES AND DISCOVERIES AND STILL SO MUCH MORE TO LEARN

The developmental neurotoxicity of legacy vs. contemporary polychlorinated biphenyls (PCBs): similarities and differences

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Abstract

Although banned from production for decades, PCBs remain a significant risk to human health. A primary target of concern is the developing brain. Epidemiological studies link PCB exposures in utero or during infancy to increased risk of neuropsychiatric deficits in children. Nonclinical studies of legacy congeners found in PCB mixtures synthesized prior to the ban on PCB production suggest that non-dioxin-like (NDL) congeners are predominantly responsible for the developmental neurotoxicity associated with PCB exposures. Mechanistic studies suggest that NDL PCBs alter neurodevelopment via ryanodine receptor-dependent effects on dendritic arborization. Lightly chlorinated congeners, which were not present in the industrial mixtures synthesized prior to the ban on PCB production, have emerged as contemporary environmental contaminants, but there is a paucity of data regarding their potential developmental neurotoxicity. PCB 11, a prevalent contemporary congener, is found in the serum of children and their mothers, as well as in the serum of pregnant women at increased risk for having a child diagnosed with a neurodevelopmental disorder (NDD). Recent data demonstrates that PCB 11 modulates neuronal morphogenesis via mechanisms that are convergent with and divergent from those implicated in the developmental neurotoxicity of legacy NDL PCBs. This review summarizes these data and discusses their relevance to adverse neurodevelopmental outcomes in humans.

Keywords Axonal outgrowth · Calcium signaling · CREB · Dendritic arborization · Neuronal morphogenesis · Neurodevelopmental disorders · Persistent organic pollutants · Ryanodine receptor

Introduction

Polychlorinated biphenyls (PCBs) are a class of 209 structurally related chemicals, or congeners, comprised of a biphenyl with a variable number of chlorine substitutions in varying positions on the benzene rings. PCBs are broadly categorized as dioxin-like (DL) or non-dioxin-like (NDL) congeners based on their three-dimensional structure and affinity for the aryl hydrocarbon receptor (AhR). DL congeners are co-planar and bind to the AhR with moderate to high affinity, whereas the NDL congeners are non-coplanar with negligible to no binding affinity for the AhR (Pessah et al. 2010) (Fig. 1).

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During the twentieth century, PCBs were synthesized and sold globally as commercial mixtures (Aroclor®, Clophen®, Phenclor®, or Kanechlor®) that varied by the percentage of chlorine by mass. While these mixtures contained both DL and NDL congeners, the specific congener profile varied between Aroclor mixtures. The chemical stability of the higher-chlorinated PCBs that predominated in these commercial mixtures made them desirable for numerous industrial and commercial applications, and also conferred resistance to environmental degradation. This environmental persistence combined with their lipophilic nature resulted in significant bioaccumulation of PCBs in food webs, including human food supplies (McIntyre and Beauchamp 2007). The realization in the 1960s that PCBs were pervasive pollutants (Jensen 1972), coupled with growing concern regarding human cancer risks associated with PCB exposure, led the United States Congress to ban PCB production in the USA in 1979. The Stockholm Convention on Persistent Organic Pollutants (POPs) instituted a more global ban on PCB production in 2001 (Carpenter 2006; White and Birnbaum 2009). Following these regulatory efforts, environmental levels

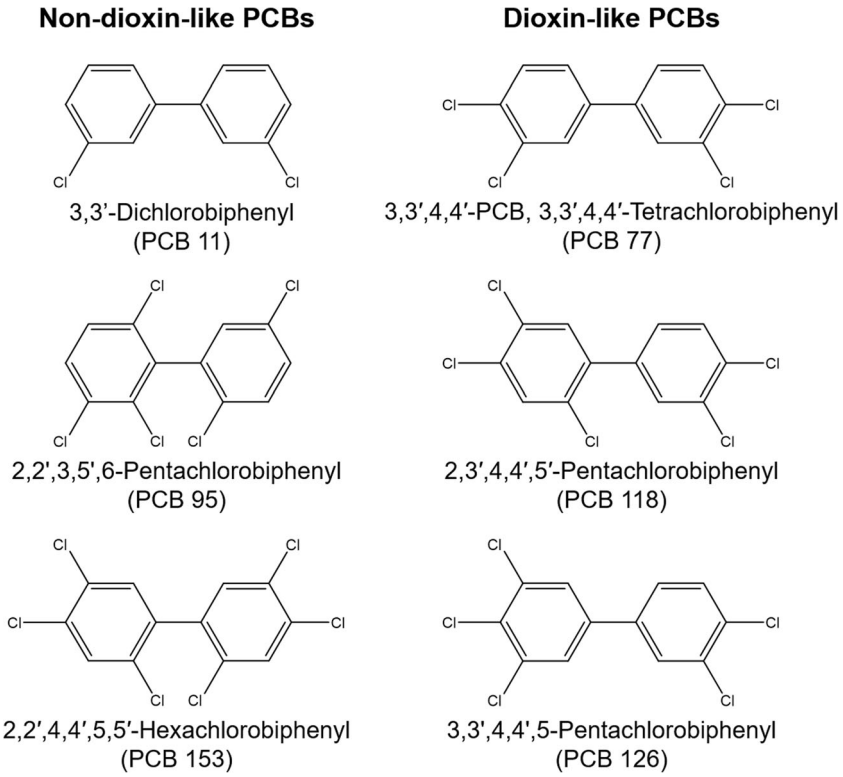

Fig. 1 Examples of non-dioxin-like (NDL) and dioxin-like (DL) PCB congeners. The higher chlorinated, legacy NDL PCBs have > 1 ortho-substituted chlorine, which creates steric hindrance, thereby preventing these congeners from assuming a coplanar configuration. NDL congeners have negligible or no activity at the AhR. Lightly chlorinated, non-Aroclor or "contemporary" PCBs like PCB 11 also have no activity at the AhR. In contrast, DL PCB congeners typically have ≤ 1 chlorine at the ortho positions of the biphenyl and are at their lowest energy state when lying in a coplanar configuration, like dioxin. Similar to dioxin, these congeners also have activity at the arylhydrocarbon receptor (AhR)

of these "legacy" PCBs present in commercial mixtures steadily decreased. However, these higher-chlorinated legacy PCBs remain a risk to human health due to continued use of old equipment containing PCBs, leaching of PCBs from hazard waste sites, and off-gassing of PCBs from aging construction materials (Consonni et al. 2012; Hopf et al. 2009; Koh et al. 2015).

Emerging evidence indicates that a significant component of contemporary human PCB exposures includes PCB congeners not present in Aroclors and other "legacy" commercial mixtures. Data collected over the last decade have documented increasing levels of "non-legacy" or "contemporary" PCB congeners in various environmental media (Hornbuckle and Robertson 2010; Hu and Hornbuckle 2010; Koh et al. 2015), including indoor and outdoor air (Hu et al. 2008) and human foods (Chen et al. 2017). These contemporary PCB congeners, many of which are more lightly chlorinated than the legacy congeners, are inadvertent byproducts of current pigment manufacturing processes. For example, PCB 11, a lightly chlorinated congener not found in Aroclors or other commercial mixtures, is generated during the synthesis of paint pigments, particularly azo/diarylide (yellow) and phthalocyanine (blue, green) pigments (Guo et al. 2014; Hu and Hornbuckle 2010; Shang et al. 2014). While congeners associated with the legacy PCB mixtures are also detected in various pigments produced using contemporary manufacturing processes, including the DL PCBs 77, 114, and 123, and the NDL PCB 95, the congeners detected with the greatest frequency in many pigments are PCB 11 and other lightly chlorinated congeners (Hu and Hornbuckle 2010). These pigments are used extensively to not only color paint, but also inks, paper, textiles, leather, plastics, and even cosmetics and food products (Gregory 2000; Stolz 2001). Of concern, studies in the USA have documented exposure to these lightly chlorinated PCBs in children and their mothers living in urban and rural areas of the Midwest (Koh et al. 2015, 2016; Marek et al. 2013). These contemporary PCB congeners have also been detected in the serum of pregnant women living in Northern California who are at increased risk of having a child diagnosed with a NDD (Granillo et al. 2019; Sethi et al. 2017a).

Here, we review the data associating PCBs with adverse neurodevelopmental outcomes, which is a primary endpoint of human health concern for these POPs (Berghuis et al. 2015; Pessah et al. 2019). This review primarily summarizes work from our laboratory that was presented at the 10th International PCB Workshop in Krakow, Poland. We examine the experimental evidence demonstrating that NDL legacy PCBs and the contemporary pollutant, PCB 11, disrupt neuronal morphogenesis via divergent and convergent mechanisms. We also discuss the relevance of these findings to human NDDs, and identify critical data gaps in the PCB developmental neurotoxicity literature.

PCB developmental neurotoxicity

PCBs first gained attention as developmental neurotoxicants following two accidental human poisonings with cooking oil

contaminated with PCBs: the Yusho incident in Japan in 1968 (Mitoma et al. 2015) and the Yu-Cheng incident in Taiwan in 1979 (Hsu et al. 1985). Infants born to women who ingested PCB-contaminated cooking oil while pregnant had a significantly increased incidence and severity of cognitive and psychomotor deficits. While these incidents involved high-level PCB exposures, subsequent epidemiological studies of infants and children exposed to lower, environmentally relevant levels of PCBs during development further suggested that PCBs are developmental neurotoxicants (Pessah et al. 2019). Multiple reviews have concluded that the epidemiological literature generally supports the hypothesis that exposure to PCBs during critical developmental periods increases the risk of adverse neuropsychological function in children, evidenced as impairments in executive function, psychomotor function, attention, learning, and memory (Berghuis et al. 2015; Pessah et al. 2019; Schantz et al. 2003). More recently, in utero exposure to PCBs has been positively associated with increased risk of NDDs, including attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) (Cheslack-Postava et al. 2013; Eubig et al. 2010; Granillo et al. 2019; Lyall et al. 2017; Pessah et al. 2019; Rosenquist et al. 2017; Sagiv et al. 2010).

A question of critical importance to assessing the risks of developmental PCB exposure is whether developmental neurotoxicity is generalizable to all PCB congeners. Exposure studies suggest that NDL PCB congeners predominate in human samples, including umbilical cord blood, breast milk, and post-mortem brain (Pessah et al. 2019). However, because a variety of analytical techniques are used for detection of PCBs in human tissues and often differing PCB congener profiles are analyzed across cohorts, it has been difficult to discern whether adverse neurodevelopmental outcomes are predominantly associated with specific subsets of PCB congeners. Nonclinical studies, which have largely focused on the legacy PCB congeners, suggest that NDL congeners mediate much of the developmental neurotoxicity associated with the legacy Aroclors and other industrial mixtures (Pessah et al. 2010; Pessah et al. 2019; Sable and Schantz 2006; Schantz et al. 2003; Winneke 2011). The question of whether DL congeners are directly neurotoxic to the developing brain remains controversial (Pessah et al. 2019), although data from animal models indicates that deficits in cognitive function do not appear to be directly driven by DL PCBs (Sable and Schantz 2006). However, other nonclinical studies suggest that DL PCBs can influence neurotoxic outcomes of NDL PCBs by inducing expression of cytochrome P450 enzymes that subsequently metabolize NDL PCBs (Curran et al. 2012; Giera et al. 2011; Klinefelter et al. 2018).

It is widely posited that PCBs alter the normal trajectory of neurodevelopment by potentially several different mechanisms, including thyroid hormone (TH) disruption, altered γ-aminobutyric acid (GABA) signaling, or interference with intracellular Ca^{2+} dynamics (Pessah et al. 2010; Pessah et al. 2019; Winneke 2011). Below, we briefly summarize the evidence of PCB action on TH and GABA signaling before providing a more extensive review of the data causally linking PCB effects on Ca^{2+}-dependent signaling to endpoints of direct relevance to human NDDS, specifically altered neuronal morphogenesis.

PCB effects on TH and GABA signaling

The scientific premise underlying the TH hypothesis of PCB developmental neurotoxicity is based on epidemiological evidence demonstrating that marked TH deficiency interferes with normal neurodevelopment (Rovet 2014) and data indicating that developmental exposures to PCBs can decrease serum TH levels in both human and animal models (Hagmar 2003; Martin and Klaassen 2010; Zoeller et al. 2002). There is nonclinical evidence linking the effects of PCBs on circulating TH levels to developmental neurotoxicity. For example, TH supplementation was found to prevent motor and auditory deficits induced by developmental exposure to Aroclor 1254 (Goldey and Crofton 1998). Additionally, in vitro data support a key role for TH disruption in mediating PCB effects on oligodendrocyte maturation and myelination (Nave and Werner 2014).

However, recent epidemiologic studies suggest that developmental PCB exposure in humans is not consistently associated with decreased serum TH levels (Itoh et al. 2018; Li et al. 2018), and nonclinical studies suggest that the cognitive deficits associated with developmental PCB exposure occur independent of decreased TH levels. For example, developmental exposures to Aroclor 1254 at 8 mg/kg/day significantly reduced serum T4 levels, but were not associated with learning and memory deficits in rats assessed using the Morris water maze or T-maze (Zahalka et al. 2001). Conversely, developmental exposure to Aroclor 1254 at 1 mg/kg/day caused performance deficits in the Morris water maze in the absence of significantly decreased serum T3 or T4 levels (Yang et al. 2009). Studies examining hippocampal neurogenesis following developmental exposure to Aroclor 1254 at 6 mg/kg/day in the maternal diet observed no effect on neuronal progenitor cell proliferation and survival despite a significant reduction in maternal serum TH (Naveau et al. 2014; Parent et al. 2016). The relevance of the doses of A1254 used in these nonclinical studies to human exposures is suggested by data indicating that total brain PCB levels in weanling rats exposed to Aroclor 1254 in at 1.0 mg/kg/day in the maternal diet ranged from 0.5 to 3.0 ng/g wet weight (Yang et al. 2009). This is well within the range of total PCB levels measured in human post-mortem brain, which range from approximately 66 ng/g wet weight in samples from Greenland (Dewailly et al. 1999) to

1.5 ng/g wet weight (range < LOD to 18.5 ng/g ww) in post-mortem samples from the USA (Mitchell et al. 2012).

These observations do not rule out thyroid hormone–dependent mechanisms other than PCB-induced hypothyroidism (Pinson et al. 2016; Wadzinski et al. 2014; Zoeller 2007). For example, gestational exposure of rats to Aroclor 1254 at 1 or 4 mg/kg/day in the maternal diet increased expression of TH-responsive genes in the fetal cortex despite significantly reducing maternal levels of serum TH, suggesting direct effects of PCBs on TH receptors in the fetal brain (Bansal et al. 2005; Gauger et al. 2004). However, others observed no effect of developmental exposure to Aroclor 1254 at 6 mg/kg/day on brain TH gene expression or genes related to TH function (Royland and Kodavanti 2008). Consistent with the latter observation, recent in vitro studies of PCBs abundant in the serum of pregnant women found no significant agonistic or antagonistic interactions with canonical TH receptors expressed in a TH reporter cell line when exposed to these PCBs singly or in combination over a wide range of concentrations (Sethi et al. 2019). This same study also saw no effect of the hydroxylated or sulfated metabolites of PCB 11 and PCB 52 on TH receptor activity. These observations are consistent with an earlier study that failed to detect a direct interaction between Aroclor 1254 and the THR (Gauger et al. 2004), and screening studies of different PCBs than those tested by Sethi et al. that used reporter cell lines expressing only the THR alpha isoform (Pencikova et al. 2018; Takeuchi et al. 2017). In contrast, other in vitro studies using TH reporter cell lines that expressed only the TH receptor β1 isoform observed agonistic activity of micromolar concentrations of hydroxylated PCB metabolites (Iwasaki et al. 2002; Miyazaki et al. 2008). There is also evidence that PCBs may affect TH signaling via mechanisms upstream of the TH receptor, such as disruption of the hypothalamic-pituitary-adrenal (HPA) axis (Zimmer et al. 2009) or modulation of crosstalk between TH and other endocrine hormones and nuclear receptors (Kouidhi and Clerget-Froidevaux 2018). However, to date, there are no experimental data directly linking PCB effect on TH signaling to effects of developmental PCB exposure on cognitive function or on neurodevelopmental processes of direct relevance to NDDs.

Experimental evidence suggests that lightly chlorinated NDL PCBs may also cause developmental neurotoxicity via allosteric modulation of the $GABA_A$ receptor. An in vitro study using *Xenopus* oocytes discovered that PCB 28 and PCB 52 interact with the $GABA_A$ receptor to potentiate GABA-induced ion current in a concentration-dependent manner at concentrations ≥ 0.3 μM or 10 μM, respectively (Antunes Fernandes et al. 2010b). PCBs 101, 138, 153, or 180 had no effect on GABA-mediated currents, suggesting this mechanism may be unique to lightly chlorinated PCBs. Interestingly, PCB 153 partially attenuated the effect of PCB 28 on $GABA_A$ receptor activity, suggesting potential mixture effects. This same group also found that the PCBs 19, 47, 51, and 100 are able to directly activate the $GABA_A$ receptor in the absence of GABA, with modulation of ion current depending on the chlorination pattern of the congeners tested (Antunes Fernandes et al. 2010a; Hendriks et al. 2010). In vivo studies demonstrated that while developmental exposure to PCB 52, 138, or 180 at 1 mg/kg/d during gestation and lactation via the maternal diet caused learning and motor deficits in rats, only PCB 52 exposure significantly increased extracellular GABA levels in the cerebellum (Boix et al. 2010).

Collectively, the experimental data identify TH and GABAergic signaling as potential targets of PCBs, but underscore the importance of congener-specific effects and potential interactions between congeners. However, TH and GABAergic signaling may not be the most sensitive mechanisms by which PCBs cause developmental neurotoxicity, as discussed below.

NDL PCBs alter synaptic connectivity via Ca^{2+}-dependent mechanisms

The spatiotemporal patterning of cytoplasmic Ca^{2+} is tightly regulated during normal neurodevelopment (Berridge 2006; Brini et al. 2014). Structure-activity relationship (SAR) studies have demonstrated that NDL PCBs (Kodavanti and Tilson 2000; Yang and Kodavanti 2001), but not DL PCBs (Do and Lee 2012), increase intracellular Ca^{2+} levels and alter Ca^{2+} signaling in primary neuronal cell cultures. As demonstrated using pharmacologic tools that block specific Ca^{2+} channels, NDL PCBs can increase levels of intracellular Ca^{2+} in neurons by activating NMDA receptors or L-type voltage-sensitive Ca^{2+} channels in the plasma membrane (Inglefield and Shafer 2000; Mundy et al. 1999), and by sensitizing ryanodine receptors (RyR) (Pessah et al. 2010) and inositol 1,4,5-trisphosphate receptors (Inglefield et al. 2001) in the endoplasmic reticulum. Of these various mechanisms, the most sensitive is RyR sensitization. Thus, long-term exposure (10–13 days) of primary cerebellar neurons to NDL PCB 52 at μM concentrations or to NDL PCBs 138, 153, or 180 at high nanomolar (nM) concentrations disrupted the glutamate-nitric oxide-cGMP pathway via activation of NMDA receptors (Llansola et al. 2010; Llansola et al. 2009). In contrast, NDL PCBs interact directly with RyRs to stabilize these channels in the open configuration over concentrations ranging from picomolar (pM) to nM to μM, depending on the RyR potency of the PCB congener (Holland et al. 2017; Samso et al. 2009). As determined using electrophysiological, biochemical, and cellular approaches, the interaction of NDL PCBs with RyRs exhibits a stringent SAR, including stereoselectivity (Feng et al. 2017; Fritsch and Pessah 2013; Holland et al. 2017; Yang et al. 2014).

Sensitization of the RyR by NDL PCBs increases the frequency and amplitude of Ca^{2+} oscillations in the somatodendritic domain of primary rat hippocampal neurons in dissociated culture (Wayman et al. 2012a), and alters the

plasticity of hippocampal CA1 neurons in acute slice culture (Wong et al. 1997). In primary mouse cortical neurons, the hydroxylated metabolite of the higher chlorinated NDL PCB 106 increases intracellular Ca^{2+} oscillations at μM concentrations, and pharmacological blockade of the RyR prevents this effect (Londono et al. 2010). In vitro studies using primary rat hippocampal neurons demonstrate that RyR sensitization by pM to nM concentrations of PCB 95, a NDL congener with potent RyR activity, activates two Ca^{2+}-dependent signaling pathways that mediate activity-dependent dendritic arborization and synapse formation during normal neurodevelopment (Wayman et al. 2008; Wayman et al. 2006) (Fig. 2). In the first signaling pathway, PCB 95 sensitization of RyRs sequentially activates CaMKK, CaMKIα/γ, MEK/ERK, and CREB to increase transcription of Wnt2, which acts as an autocrine factor to promote dendritic growth (Wayman et al. 2012a). In the second pathway, PCB 95 activates CREB to upregulate transcription of miR132, which then suppresses translation of p250GAP mRNA. The resulting decrease in p250GAP promotes synaptogenesis, evident as increased density of dendritic spines and increased frequency of miniature excitatory postsynaptic currents (Lesiak et al. 2014).

Several lines of evidence support a causal link between NDL PCB effects on RyR sensitization and promotion of dendritic growth. First, RyR-active PCBs 95 and 136 promote dendritic arborization and synaptogenesis in primary neurons; in contrast, PCB 66, which has physicochemical properties similar to that of PCB 95, but lacks RyR activity, has no significant effect on dendritic morphology (Wayman et al. 2012b; Yang et al. 2014). Second, siRNA knockdown or pharmacological blockade of RyRs inhibits the dendrite- and spine-enhancing activity of PCBs 95 and 136 (Lesiak et al. 2014; Wayman et al. 2012b; Yang et al. 2014). Third, several RyR-active PCBs are chiral, including PCB 136. PCB 136 atropselectively sensitizes RyRs (Pessah et al. 2009) and demonstrates the same atropselectivity with respect to its effects on dendritic arborization in vitro (Yang et al. 2014). RyR-active NDL PCBs promote dendritic growth in not only rat hippocampal neurons but also mouse hippocampal neurons, and cortical neurons derived from mice or rats (Keil et al. 2019; Wayman et al. 2012b). Furthermore, the morphogenic effects of NDL PCBs are dendrite-selective and they have not been observed to alter axonal growth (Yang et al. 2014). In vitro studies of PCB-induced dendritic growth have consistently revealed a non-monotonic or inverted U-shaped concentration-effect relationship, with dendrite-promoting activity observed in the pM to nM range but not at femtomolar (fM) or μM concentrations (Wayman et al. 2012b; Yang et al. 2014). The biological mechanism(s) contributing to this non-linear concentration-effect relationship are unknown, but are not due to cytotoxicity at the higher concentrations (Wayman et al. 2012b; Yang et al. 2014).

Changes in dendritic growth in response to neural activity (aka "experience"), are considered the biological substrate of associative learning (Pittenger and Kandel 2003). Altered patterns of dendritic arborization during development are associated with neurobehavioral deficits in animal models (Berger-Sweeney and Hohmann 1997) and in humans (Copf 2016; Penzes et al. 2011; Supekar et al. 2013). Thus, it is biologically plausible that

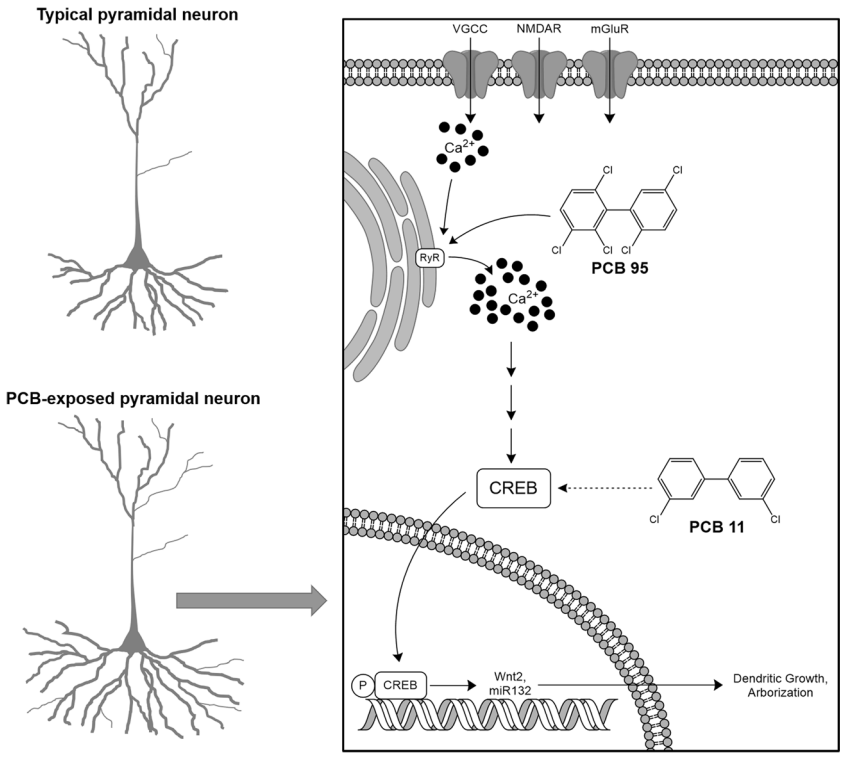

Fig. 2 Schematic illustrating PCB effects on dendritic arborization. Data available in the peer-reviewed literature indicate that developmental exposure to higher chlorinated NDL PCB congeners, exemplified by PCB 95, or the lightly chlorinated contemporary congener, PCB 11, enhances dendritic arborization in pyramidal neurons of the hippocampus and cortex. PCB 95 and 11 act on different proximal molecular targets, but converge on the CREB signaling pathway. Ca^{2+}, calcium; CREB, cAMP response element binding protein; RyR, ryanodine receptor; VDCC, voltage-dependent calcium channel

PCB effects on dendritic arborization contribute to PCB developmental neurotoxicity. In support of this hypothesis, learning and memory are impaired in rats exposed to Aroclor 1254 at 1, but not 6, mg/kg in the maternal diet (Yang et al. 2009). These behavioral deficits coincided with increased RyR activity, increased basal dendritic arborization, and altered dendritic plasticity in brain regions known to be important for performance in the Morris water maze (Yang et al. 2009). This study also demonstrated similar non-monotonic dose-response relationships for the behavioral effects, dendritic effects and RyR sensitization of developmental Aroclor exposure. However, Aroclor effects on serum levels of TH or sex steroids exhibited distinctly different dose-response relationships (Yang et al. 2009). In a separate study, developmental exposure to PCB 95 in the maternal diet similarly enhanced the dendritic arborization of hippocampal CA1 pyramidal neurons in a non-monotonic dose-related manner (Wayman et al. 2012b).

PCB 11 modulates neuronal morphogenesis via CREB-dependent mechanism(s)

In contrast to legacy NDL PCBs, there is a paucity of data regarding the potential developmental neurotoxicity of the contemporary lightly chlorinated NDL PCBs. This gap is significant in light of data from recent studies indicating that mothers who are at increased risk of having a child diagnosed with a NDD (Hertz-Picciotto et al. 2018) had elevated levels of lightly chlorinated PCBs in their serum (Granillo et al. 2019; Sethi et al. 2018). In one of these studies, the lightly chlorinated contemporary congener, PCB 11, was detected in all 241 women enrolled in the study at concentrations ranging from 0.005 to 1.717 ng/mL. It ranked as second in abundance to PCB 28, another lower chlorinated PCB, and together, PCB 11 and PCB 28 constituted more than 50% of the total PCB mass in these samples (Sethi et al. 2019).

In vitro studies of primary hippocampal and cortical neurons revealed that PCB 11 and its hydroxylated and sulfated metabolites, which are found in human serum (Grimm et al. 2015; Grimm et al. 2017), significantly alter neuronal morphogenesis in a species-, sex-, and brain region–specific manner (Sethi et al. 2017b; Sethi et al. 2018). Similar to the legacy NDL PCBs, PCB 11 enhanced dendritic arborization; however, in contrast to the legacy NDL PCBs, PCB 11 also promoted axonal growth. PCB 11 elicited these morphogenic effects at concentrations as low as 1 fM (approximately 0.22 ng/mL), which is within the range of PCB 11 concentrations observed in the serum of pregnant women. The data from the Sethi et al. (2018) study suggested PCB 11 is more potent than the higher chlorinated legacy congeners PCB 95 or PCB 136 in promoting dendritic arborization, since the lowest concentrations at which these legacy congeners significantly enhanced dendritic arborization (low pM range) were an order of magnitude above the lowest observed effect level for PCB 11. These studies also suggested that the sensitivity to the morphogenic effects of PCB 11 and its metabolites varied between neuronal cell types. For example, the axon-promoting activity of OH-PCB 11 and the dendrite-promoting activity of PCB 11 were significant at lower concentrations in cortical than hippocampal neurons, whereas PCB 11–induced axonal growth was significant at lower concentrations in hippocampal neurons relative to cortical neurons. In general, the PCB 11 sulfate metabolite was more potent than either PCB 11 or hydroxylated PCB 11, suggesting that PCB 11 metabolism is as much a toxifying as detoxifying mechanism.

Mechanistic studies of PCB 11–induced dendritic growth revealed that PCB 11 does not modulate dendritic arborization via interaction with canonical molecular targets of legacy DL or NDL PCBs. Specifically, PCB 11 did not activate the AhR (Sethi et al. 2018), the TH receptor (Sethi et al. 2019), or the RyR (Holland et al. 2017). Moreover, pharmacologic blockade of AhR, THR, or RyR did not inhibit the dendrite-promoting effects of PCB 11 (Sethi et al. 2018). Pharmacologic block of L-type $Ca2^{+}$ channels or the IP_3 receptor also had no significant effect on dendritic growth in primary neurons exposed to PCB 11 (Sethi et al. 2018). While PCBs have been shown to increase intracellular levels of reactive oxygen species (ROS) (Winneke 2011), and ROS is known to modulate dendritic arborization (Chandrasekaran et al. 2015), antioxidants did not inhibit PCB 11–induced dendritic growth in vitro (Sethi et al. 2018). However, siRNA knockdown or pharmacologic inhibition of CREB significantly decreased PCB 11–induced dendritic arborization (Sethi et al. 2018) (Fig. 2). The molecular initiating event(s) of PCB 11–induced dendritic growth upstream of CREB activation remain to be determined.

While it is yet unknown whether developmental exposures to PCB 11 similarly modulate dendritic arborization in vivo, these findings suggest that the higher chlorinated legacy NDL PCBs and the lightly chlorinated contemporary PCB congeners, at least as exemplified by PCB 11, have shared (dendrite-promoting) and unique (axon-promoting) effects on neuronal morphogenesis (Table 1). Interestingly, while the mechanism(s) mediating the dendrite-promoting activity of legacy NDL PCBs and contemporary lower chlorinated PCBs converge on CREB signaling, the upstream signaling events that link PCBs to CREB activation are divergent with legacy NDL PCBs triggering CREB-dependent signaling via RyR-dependent mechanisms and PCB 11 activating CREB via RyR-independent mechanisms (Fig. 2 and Table 1).

The relevance of PCB effects on neuronal morphogenesis to human NDDs

The organizational patterning of synaptic connections that occurs during development is a critical determinant of cognitive function

Table 1 Comparison between legacy and contemporary PCB effects and mechanisms

Parameter	PCB 95	PCB 11
Present in serum of women at risk for having a child with a NDD	✓	✓
Effects on dendritic arborization in primary neurons of the developing brain	↑	↑
Effects on axonal outgrowth in primary neurons of the developing brain	No effect	↑
Ryanodine receptor activity	+++	Negligible activity
CREB-dependent effects on dendritic growth	✓	✓

CREB, cAMP response element-binding protein

later in life (Copf 2016). Synaptic connectivity is determined in part by the rate and extent of dendritic and axonal growth (Libersat and Duch 2004; Scott and Luo 2001), and disruptions in the timing or magnitude of axonal and dendritic growth can perturb the pattern of connections formed between neurons (Berger-Sweeney and Hohmann 1997). Moreover, altered dendritic and axonal morphology are consistent pathologic correlates of the clinical symptoms associated with diverse NDDs (Copf 2016; Engle 2010; Penzes et al. 2011; Supekar et al. 2013). Thus, synaptic connectivity likely represents a convergence point in pathogenic mechanisms that confer NDD risk (Stamou et al. 2013) (Fig. 3).

The convergence of legacy and contemporary NDL-PCBs on CREB signaling has important implications in the context of PCB developmental neurotoxicity (Fig. 2). CREB is a key transcriptional regulator of dendritic growth in response to diverse stimuli, including activity (Redmond et al. 2002; Wayman et al. 2006). Therefore, the inappropriate activation of this transcriptional pathway as a result of PCB exposure may have significant functional consequences in the developing brain, including cortical overgrowth and hyperconnectivity, which are phenotypes observed in ASD and other neurodevelopmental disorders (Stamou et al. 2013). Consistent with this suggestion, mutations in CREB and/or CREB signaling are implicated in the pathogenesis of numerous NDDs (Bu et al. 2017; D'Andrea et al. 2015; Ngounou Wetie et al. 2015; Todd and Mack 2001). These observations, together with clinical evidence indicating that altered dendritic complexity is a common pathologic feature of diverse NDDs (Alaerts et al. 2016; Copf 2016; Supekar et al. 2013), suggest the human relevance of experimental evidence that developmental PCB exposure alters the dendritic complexity of developing neurons.

Data gaps and directions for future study

A critical data gap is the paucity of data regarding the developmental neurotoxicity of the contemporary non-Aroclor PCB congeners. Evidence of increasing human exposure to these congeners (Hornbuckle and Robertson 2010; Hu and Hornbuckle 2010; Koh et al. 2015; Sethi et al. 2019) underscores the need to address this data gap. There is also a need to determine which of the persistent legacy NDL PCBs to which humans are exposed constitute the greatest risk to the developing human brain, and to identify genetic polymorphisms that modify individual susceptibility to PCB developmental

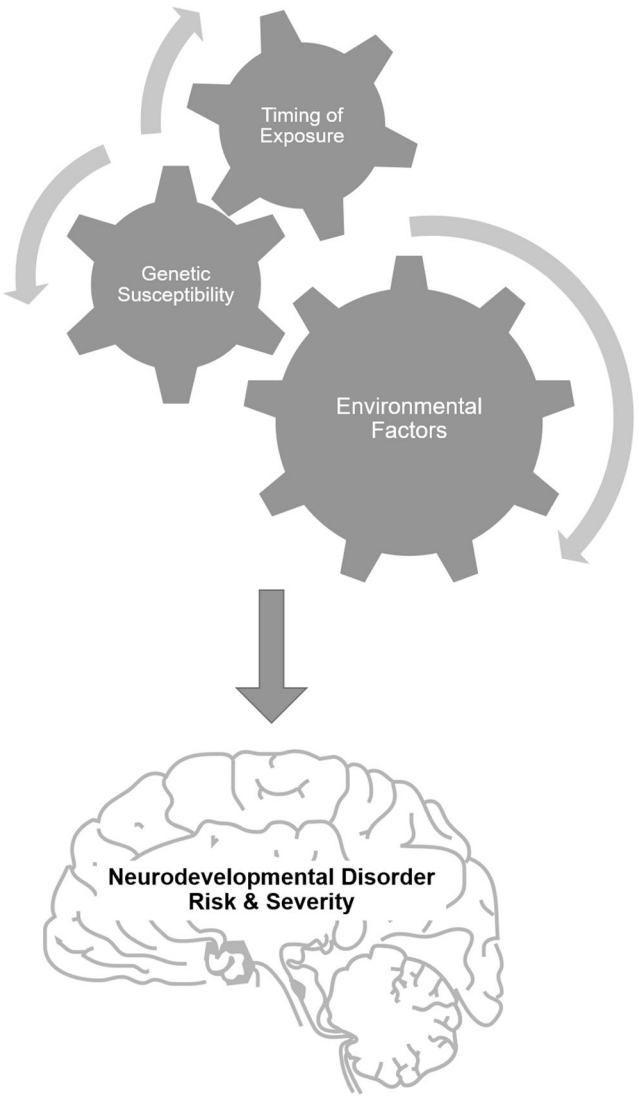

Fig. 3 Factors that influence the risk and/or severity of NDDs. Accumulating evidence indicates that individual risk for NDDs is determined by a complex interplay of genetic risk factors that confer susceptibility, exposure to environmental stressors, including neurotoxic chemicals, and the timing of environmental exposures. Exposures that occur during critical neurodevelopmental windows pose a greater risk to the developing brain

neurotoxicity. Given recent data indicating that hydroxylated and sulfated PCB metabolites are also potent drivers of neurotoxic effects, further research into PCB metabolic fate and the actions of PCB metabolites is also warranted. In light of data documenting significantly elevated levels of airborne PCBs, including PCBs 11 and 95, in schools (Thomas et al. 2012) and outdoor environments (Hu et al. 2008), another critical data gap is the lack of information regarding the relative neurotoxic impact of diet vs. inhalation as routes of PCB exposure (Ampleman et al. 2015; Lehmann et al. 2015). Addressing these data gaps will be critical for rigorously assessing the risks that PCBs pose to the developing human brain.

To address these data gaps, it will be important to implement a number of experimental approaches. First is an urgent need for comprehensive analyses of the PCB congener profile that comprises current human exposures. Epidemiological studies need to move away from the typical practice of measuring a single or small subset of PCB congeners as indicators of cumulative PCB exposure (Longnecker et al. 2003). Further, many human studies have focused on DL congeners, despite evidence from animal studies suggesting that DL PCBs are likely not responsible for many of the cognitive and behavioral abnormalities observed in humans (Bernhoft et al. 1994; Bushnell and Rice 1999; Schantz et al. 1996). Future human studies should endeavor to quantify all 209 PCB congeners or at least include an increased number and wider variety of PCB congeners in exposure assessments to gain a more comprehensive understanding of contemporary human exposures. Human studies should also leverage emerging mechanistic data from experimental studies to stratify cohorts by relevant genetic factors that may modify risk of PCB developmental neurotoxicity (Granillo et al. 2019).

Nonclinical studies need to move away from using industrial Aroclor PCB mixtures because these do not model congener profiles relevant to current human exposures (Frame et al. 1996; Koh et al. 2015; Longnecker et al. 2003; Sethi et al. 2018; Sethi et al. 2019). Rather, researchers should use PCB congeners and mixtures that mimic the contemporary congener compositions and levels observed in human sera, placenta, and/or cord blood to better model PCB exposures in the gestational environment. There is also need for nonclinical studies that establish causal relationships between molecular, cellular, and behavioral endpoints. Behavioral assessments should focus on specific domains previously identified as targets of PCB exposure in epidemiological studies, which are primarily executive function and cognitive flexibility. In vitro and alternative models should be developed to more rapidly screen legacy and contemporary PCB congeners, both individually and as mixtures, to establish relative potencies and mechanistic convergence and divergence. Results from such screens will be useful for prioritizing PCB congeners to test in animal models and will inform interpretation of results generated using PCB mixtures in vivo.

Conclusions

PCBs remain a continuing environmental health concern, although recent data suggest that the profile of PCB congeners of concern may have shifted over the past several decades. Contemporary human exposures are increasingly predominated by legacy NDL PCBs and lightly chlorinated non-Aroclor PCBs. Mechanistic data indicate that NDL PCBs alter normal trajectories of neurodevelopment by modulating dendritic arborization, while recent in vitro data suggest lightly chlorinated contemporary PCBs influence neurodevelopment by modulating both dendritic and axonal growth. Interestingly, while the proximal signaling events mediating the dendritic effects of these two groups of PCBs are divergent, with NDL PCBs enhancing dendritic growth via RyR-dependent mechanisms, and PCB 11 influencing dendritic growth via RyR-independent mechanisms, both converge on CREB signaling. These observations suggest that CREB signaling may be a relevant target for stratifying epidemiological studies of PCB developmental neurotoxicity, and for setting up high throughput screens using CREB signaling as the relevant outcome to identify those PCBs that pose the greatest risk to the developing human brain.

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Compliance with ethical standards

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