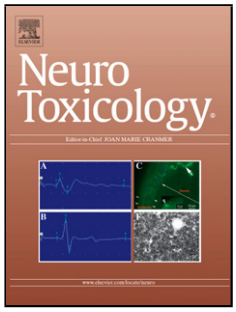

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Developmental Exposure to an Environmental PCB Mixture Delays the Propagation of Electrical Kindling from the Amygdala

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ABSTRACT

Developmental PCB exposure impairs hearing and induces brainstem audiogenic seizures in adult offspring. The degree to which this enhanced susceptibility to seizure is manifest in other brain regions has not been examined. Thus, electrical kindling of the amygdala was used to evaluate the effect of developmental exposure to an environmentally relevant PCB mixture on seizure susceptibility in the rat. Female Long-Evans rats were dosed orally with 0 or 6 mg/kg/day of the PCB mixture dissolved in corn oil vehicle 4 weeks prior to mating and continued through gestation and up until postnatal day (PND) 21). On PND 21, pups were weaned, and two males from each litter were randomly selected for the kindling study. As adults, the male rats were implanted bilaterally with electrodes in the basolateral amygdala. For each animal, afterdischarge (AD) thresholds in the amygdala were determined on the first day of testing followed by once daily stimulation at a standard 200 μA stimulus intensity until three stage 5 generalized seizures (GS) ensued. Developmental PCB exposure did not affect the AD threshold or total cumulative AD duration, but PCB exposure did increase the latency to behavioral manifestations of seizure propagation. PCB exposed animals required significantly more stimulations to reach stage 2 seizures compared to control animals, indicating attenuated focal (amygdala) excitability. A delay in kindling progression in the amygdala stands in contrast to our previous finding of increased susceptibility to brainstem-mediated audiogenic seizures in PCB-exposed animals in response to a an intense auditory stimulus. These seemingly divergent results are not unexpected given the distinct source, type, and mechanistic underpinnings of these different seizure models. A delay in epileptogenesis following focal amygdala stimulation may reflect a decrease in neuroplasticity following developmental PCB exposure consistent with reductions in use-dependent synaptic plasticity that have been reported in the hippocampus of developmentally PCB exposed animals.

Keywords: PCBs, amygdala kindling, seizures

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of halogenated aromatic hydrocarbons that were primarily used as dielectric or coolant fluids in transformers, capacitors and large electrical motors. Even though banned from production in North America in the late 1970's, PCBs are still a ubiquitous environmental pollutant. They have bioaccumulated and biomagnified through the food chain and readily accumulate in the adipose tissue of humans and wildlife (Crinnion, 2011). Furthermore, continued exposure to PCBs can occur through PCBs that are found in caulking material of older buildings, including schools (Ampleman *et al*., 2015) and new evidence indicates that PCBs are produced inadvertently during the manufacture of paint pigments (Hu and Hornbuckle, 2010; Anezaki *et al*., 2014). PCBs are lipophilic and can be mobilized from the adipose tissue of the mother and enter the fetus and newborn through the placenta and through breast milk (Jacobson *et al*., 1984). PCB exposure during development has been shown to impair both neuroplasticity in forebrain structures and cognitive processes such as working memory that rely on these structures (Gilbert and Liang, 1998; Ozcam *et al*., 2004; Gilbert 2003; Carpenter *et al*., 2002) and to cause peripheral sensory deficits in hearing (Trnovec *et al*., 2010; Powers *et al*., 2006).

Recently our laboratory found that developmental exposure of rats to PCBs caused an increase in audiogenic seizure (AGS) susceptibility in adulthood (Poon *et al*., 2015; Bandara *et al*., 2016). Adult offspring of dams administered PCBs during gestation and lactation exhibited increased AGS incidence and severity compared to controls upon exposure to a 100 dB SPL noise. Two other studies have assessed seizure susceptibility following developmental PCB exposure, but the results are inconsistent. Overman *et al*, (1987) reported attenuation of seizures induced by electroconvulsive shock in adult offspring of rat dams exposed to Aroclor 1254 at 2.5 and 26 ppm (approximately 0.05-0.075 mg/kg/day or 0.52-0.78 mg/kg/day of Aroclor 1254, respectively). In contrast, developmental exposure to PCB congener 95 (1 or 6 mg/kg/day) resulted in shorter latencies to myoclonus and tonic clonic seizures as well as higher seizure scores in response to flurothyl inhalation and pentylenetetrazol kindling (Lein *et al*., 2010). Although both of these latter studies in Lein *et al*., (2010) suggest enhanced seizure

susceptibility in animals exposed to PCBs there is a relative lack of research systematically evaluating the convulsive effects of PCBs in brain regions outside the auditory brainstem.

Electrical kindling is a well-recognized animal model of temporal lobe epilepsy where repeated subthreshold electrical stimulation of the brain leads to the progressive development of generalized seizures (Goddard *et al*., 1969; Racine *et al*, 1983). In the kindling model, animals progress through several well characterized and increasingly severe behavioral and electrographic stages. In the classical kindling paradigm repeated brief, low intensity electrical stimuli are delivered through chronically implanted electrodes to any one of a number of brain areas, although the amygdala is the most common site. A brief electrographic seizure discharge (afterdischarge, AD) appears initially in response to an electrical stimulus pulse, and with once daily repetition, this AD increases in duration and amplitude (Figure 1). With sufficient stimulation, ADs evolve in the non-stimulated amygdala followed by secondary and tertiary ADs. Subsequent induction in the contralateral amygdala indicates the gradual spread of aberrant ADs to brain regions outside of the original seizure focus. Although the AD appears initially in the absence of behavioral changes, as the AD increases in complexity, the animal progresses through a series of behavioral phases (Racine, 1972) that begin as very mild facial automatisms and grow in duration and intensity over successive sessions to culminate in generalized tonic-clonic seizures. Both behavioral and electrographic parameters of convulsive activity are used as measures of the development of epileptogenesis. Once kindling has been established, the increased sensitivity to an initially relatively benign stimulus pulse is permanent (Goddard *et al*., 1969; Racine, 1972).

Not only is kindling a widely accepted model of epileptogenesis it has also been applied in toxicological studies to assess the effects of acute toxicant exposure on use-dependent plasticity (Gilbert, 1988; Gilbert and Mack, 1989; Gilbert and Mack, 1995; Gilbert and Llorens, 1993). In addition, transient developmental exposures to some toxicants have been shown to cause long lasting effects on amygdala kindling susceptibility in adulthood. An early study that assessed neonatal exposure to lindane found that rats exposed to lindane during development kindled faster and had longer and more

severe seizures compared to controls (Albertson *et al*., 1985). In contrast, developmental exposure to the toxin β, β'-iminodipropionitrile (IDPN) which causes redistribution of neurofilaments in axons (Fink *et al*., 1986) led to protracted AD development and slowed the progression of kindling in the amygdala when tested in adulthood (Gilbert and Llorens., 1993). Together, these studies indicate that developmental exposure to toxicants may cause permanent changes to neural systems that underlie amygdala kindling.

The present study was conducted to determine if an enhanced seizure sensitivity is induced by developmental PCB exposure in rats using the electrical kindling model; a model of epileptogenesis that is distinct from the audiogenic seizure model and primarily targets the limbic system as opposed to the auditory brainstem. It was hypothesized that similar to the auditory brainstem where we saw an increase in the incidence and severity of AGS in developmentally PCB exposed animals, these animals would kindle with fewer stimulations and exhibit longer ADs compared to controls.

2. MATERIALS AND METHODS

2.1 Animals.

Primiparous female Long-Evans rats, approximately 8-10 weeks of age, were purchased from Harlan (Indianapolis, IN). They were individually housed in standard polycarbonate plastic shoebox cages with wood-chip bedding, and fed rat chow (Harlan Teklad rodent diet (W) 8604) and water ad libitum. All rats were housed in a temperature- and humidity-controlled room (22°C, 40–55% humidity), on a 12/12-hr light cycle (lights on at 0700 hr). The rats were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the National Institutes of Health (2002) and National Research Council (2003).

2.2 Exposure.

The female rats were randomly assigned to exposure groups and given one of two treatments consisting of corn oil vehicle or PCBs in corn oil. Exposure began 28 days prior to breeding and continued until weaning of the pups on postnatal day (PND) 21. The PCB mixture (Fox River PCB mixture) was formulated to mimic the congener profile found in walleye fish from the Fox River in northeast Wisconsin. The mixture consisted of 35% Aroclor 1242 (Monsanto lot KB 05-415; St. Louis, MO) 35% Aroclor 1248 (AccuStandards lot F-110; New Haven, CT), 15% Aroclor 1254 (Monsanto lot KB 05-612), and 15% Aroclor 1260 (AccuStandards lot 021-020) (Kostyniak *et al*., 2005). The dose of 6 mg/kg/day was selected based on previous ototoxicity and AGS studies as a dose that impaired auditory function and increased AGS incidence and severity but did not cause overt clinical toxicity in the rats (see Kostyniak *et al*. 2005; Powers *et al*. 2006; Poon *et al*., 2015). The PCB mixture diluted in corn oil (Mazola) or the corn oil vehicle alone was pipetted onto one-half of a vanilla wafer cookie (Keebler Golden Vanilla Wafers) at a volume of 0.4 mL/kg. To arrive at a dose of 6 mg/kg and a dosing volume of 0.4 mL/kg, the PCB solution was mixed at a concentration of 15 mg/ml. The PCB and vehicle treated cookies were fed to the female rats daily with the amount of dosing solution applied to the cookies adjusted daily to account for weight gain.

2.3 Breeding, pregnancy, and weaning.

After four weeks of PCB exposure, each female was paired with an unexposed male Long-Evans rat (Harlan, Indianapolis, IN) in a hanging wire cage for 8 consecutive days with food and water ad libitum. The females were returned to their home cages each day for PCB dosing. The females were monitored for the presence of a sperm plug in order to establish gestational day 0.

On the first day after birth (PND1), the pups were examined for abnormalities, sexed and weighed. On PND 2, the litters were culled to 10 pups (five males and five females when possible), and litters with at least 7 pups had extra pups cross-fostered into them from the same treatment group to bring the litters to 8–10 pups. Cross-fostered pups were ear marked and not used for the experiment. There were 17 successful litters. Of the remaining dams, 9 were not pregnant and 4 had litters too small to be included in the study (<7 pups). Overall the non-pregnant dams and dams with small litters

were evenly distributed across the treatment groups. Dosing continued until the pups were weaned on PND 21. Two males from each of the successful litters were allowed to mature to adulthood at which time they were used for the kindling experiment. Males were chosen because a wealth of information on the progression of kindling is available and has been conducted in male rats. Pups from each litter were weighed individually on PND 2, 7, 14 and 21 days of age and then prior to kindling surgery as adults (≥ 90 days of age).

2.4 *Kindling surgery*.

Between the ages of 90-120 days four animals per day, with two animals chosen randomly from each dose group (N = 34 total), were assigned for surgery and anesthetized with isoflourane (1-5%) and mounted in a stereotaxic frame. Electrodes constructed of gold plated amphenol pins (Industrial Amphenol™) crimped in twisted strands of teflon-coated stainless steel wire (each strand 125/µm, insulated except for the tips with .5mm tip separation). Electrodes were slowly lowered in the basolateral amygdala (2.5 mm posterior to bregma, 4.5 mm lateral to midline and 7.5 mm ventral to dura) of both hemispheres and cemented in place. The amphenol pins were inserted into a 9-pin connector, which was cemented to the animal's skull and anchored with stainless steel screws. The animals were grounded through a screw inserted in the skull overlying the anterior neocortex.

2.5 *Kindling procedure*.

Two weeks following surgery, animals were placed in a plexiglass testing chamber and connected to the stimulating/recording apparatus via a noise shielded cable. The electroencephalogram (EEG) was recorded on a Windows 7 PC with National Instruments data collection hardware (PCI-6025E) and custom software written by EPA personnel. The pre-stimulation baseline, stimulation initiation and evoked afterdischarge (AD) were recorded (5 kHz sampling rate; Figure 1). An AD was defined as EEG activity lasting 5 sec or longer, consisting of spikes at a frequency of 1 Hz or greater and with an amplitude at least four-fold the size of the pre-stimulation baseline (Figure 1A). The stimulus was delivered by a Grass S-88 stimulator and two PSIU constant current converters. The

stimulus itself consisted of a 1 second train of 60 Hz biphasic square waves, each wave 1 msec in duration. For AD threshold (ADT) testing, starting with the left hemisphere, a series of stimulations were delivered beginning at 25 µA and increasing in 25 µA steps at 5 minute intervals between stimulations until an AD was observed. The same procedure was followed to determine ADT for the right hemisphere, and the hemisphere with the lower ADT was selected as the kindling site. Thereafter, a standard 200 µA stimulus was delivered once daily to the selected hemisphere until fully generalized seizures were observed (Figure 1C). Severity of the behavioral seizure and duration of the evoked AD were recorded in response to each stimulation. Behavioral seizures were scored as shown in table 1, according to the Racine scoring system (Racine, 1972).

Seizure stage	Behavioral endpoint
1	Motor arrest, facial automatism and chewing
2	Chewing and head nodding
3	Chewing, nodding and unilateral forelimb clonus
4	Rearing with bilateral forelimb clonus
5	Rearing with bilateral clonus and loss of postural control

Table 1. Behavioral endpoints corresponding to seizure stage following daily stimulation of the basolateral amygdala.

Upon completion of kindling, animals were given an overdose (up to 0.5 ml) of ketamine/xylazine; 87 $mg.kg^{-1}$/13 $mg.kg^{-1}$ and perfused through the heart with saline followed by 4% paraformaldehyde. The brains were removed and prepared for histological analysis. Coronal sections were cut at 100 µm on a cryostat, mounted and stained with methyl blue to verify electrode placement.

2.6 *Statistical analysis*.

All statistical analyses were conducted using SPSS for MS Windows (version 20.0; IBM SPSS Statistics with Exact Tests Module) with statistical significance set at $p<0.05$. Kindling rate and AD duration development were assessed using repeated measures ANOVA. The ADT, duration of the first

AD, duration of and latency to the onset of clonus for the first generalized seizure were analyzed by Students T-test.

3. RESULTS

3.1 *Developmental effects*

There were no overt signs of clinical toxicity in the dams from either the control or PCB dose group. In the PCB exposed pups, there were significant ($p<0.05$) decreases of 8, 13 and 19% in body weight compared to controls at postnatal days 7, 14 and 21. No other gross developmental abnormalities were noted. Body weight measures taken prior to and after the kindling procedure indicate that developmentally PCB exposed animals recovered to control body weights at adulthood (> PND 90).

3.2 *Basolateral amygdala electrode placement*

Thirty-four male rats, two from each litter were used to ensure sufficient number of animals/group after attrition from headcap loss and/or incorrectly placed electrodes. Data were analyzed for 21 of these animals from 8 PCB exposed and 9 control litters that had histologically confirmed correct electrode placement in the basolateral amygdala and maintained the integrity of the headcap assembly for the duration of the experiment (electrode placements for both control and PCB animals are shown in Figure 2). In litters with both animals with confirmed correct electrode placement, average values for kindling development were reported.

3.3 *Kindling Development*

The ADT did not differ between the control and PCB treated groups (Figure 3). The rate of behavioral kindling development, however, was significantly prolonged in animals exposed to PCBs. The mean number of stimulations (± SEM) required to evoke the first stage 5 seizure for PCB animals was 16.4 ± 2.0 compared to 11.5 ±0.7 in oil treated control animals (Figure 4a). To assess differences in kindling rate, a repeated measures ANOVA of treatment effects and seizure stage revealed

significant effects of treatment, [F(1,14)=8.071, p=0.013] and stage [F(4,11)=34.918, p<0.001] and a trend for treatment x stage interaction, [F(4,11)=2.725, p=0.085] (Figure 4b). The delay in kindling development induced by developmental PCBs can be further characterized by displaying it as the mean number of sessions in each stage of motor seizure. A mixed between-within subjects ANOVA of treatment and days in seizure stage revealed that there was a significant effect of treatment, [F(1,14)= 5.755, p=0.031], which Bonferroni pairwise comparison reveal is driven mainly by developmentally PCB exposed animals showing an increase in the mean number of sessions in stage 1. There was also a significant effect of stage [F(5,10)=27.042, p<0.001] where a delay in the progression (larger number of sessions were spent in the early kindling stages 0-2 compared to later stages 3-5) and no significant interaction between treatment x stage (Figure 4c). Also measured were the latency to onset of clonus and total duration of clonus at first stage 4/5 clonic seizure. These measures did not show a statistically significant difference between the control and the PCB dose groups (Figure 5).

There was a significant effect of treatment on primary AD summed across all sessions from ADT to the first stage 5 seizure where PCB exposed animals had longer cumulative AD's compared to controls, Figure 6A, [t(14)= 2.493, p=0.026)]. Similarly, assessment of the total duration of AD (primary + secondary/tertiary AD) events across sessions from ADT to first stage 5 seizure was also significant [t(14)= 2.401, p=0.031)] where PCB animals had longer total ADs compared to controls (Figure 6B). Longer cumulative ADs parallel increased number of sessions required to induce the kindled state in PCB exposed animals. The mean AD duration evoked by each stimulation however, did not differ between groups (Figure 7a and 7b) suggesting that although focal recorded ADs were similar in duration in controls and PCBs, the ADs in PCB exposed animals were less effective in propagating from the site of origin.

4. DISCUSSION

Developmental PCB exposure delayed the development of the behavioral manifestations of amygdala kindling. This delay was restricted to the initial seizure stage; once PCB animals reached

stage 2, they progressed through the remaining seizure stages at comparable rates. During stage 1 seizures, motor arrest at the time of stimulation, facial automatisms and chewing are observed which are thought to be induced by seizure activity within the immediate region of the stimulation site (amygdala) or in its direct projections (reviewed by Racine 1978). No significant differences were seen on AD threshold (both hemispheres tested), nor on the progressive increase in AD duration across sessions between control and PCB exposed animals. Significantly longer cumulative primary AD and total AD durations collapsed across all sessions were seen in PCB-treated animals compared to controls. These differences in cumulative AD parameters could be attributable to increased number of sessions required for PCB animals to reach stage 5 kindling state.

Currently there is no active research exploring a relationship between developmental PCB exposure and seizure susceptibility in humans, and there is limited research addressing the role of PCB exposure in modulating seizure susceptibility in animal models. Furthermore, the mechanism of action of PCBs on neuronal excitability is poorly understood, with some studies showing an increase and others showing a decrease in the incidence of seizure in rodents (Lein et al., 2010; Overman et al., 1987). Our own studies have indicated that developmental PCB exposure increases the incidence of AGS in offspring as adults (Poon *et al*., 2015), and this is associated with a reduction in the concentration of glutamic acid decarboxylase (GAD, an enzyme critical for synthesis of the inhibitory neurotransmitter GABA) in the inferior colliculus (IC). It was hypothesized that reduced GAD leads to reduced synthesis of GABA and a loss of inhibition in the colliculus, and therefore contributes to the induction of AGS (Bandara *et al*., 2016). Based on these results it was hypothesized that if compromise of inhibitory neurotransmission was broadly distributed throughout the brain, enhanced seizure susceptibility would not be limited to the auditory system. However, our findings with the electrical kindling model indicated the reverse: developmental exposure to PCBs delayed the behavioral progression of kindling in the basolateral amygdala relative to the control group.

One explanation for the opposing observations of enhanced brainstem but suppressed forebrain seizure responsiveness may be a focal decrease in inhibition at the IC site. It is well established that an

imbalance between excitation and inhibition in the mammalian brainstem can lead to convulsions (Faingold, 1999). Changes in the level of excitation in brain regions outside the amygdala, including the auditory brainstem, can influence amygdala excitability (Doron and LeDoux, 1999; Feng and Faingold 2002; Fritsch *et al*., 2009: Peruzzi *et al*., 1997). We have shown that developmental PCB exposure leads to cochlear outer hair cell dysfunction (Powers et al., 2006) and reductions in GAD concentrations in the IC potentially leading to a damping of inhibitory tone (Bandara et al., 2016). No such changes in GAD were seen in in the somatosensory cortex, hippocampus (Bandara et al., 2016), or in the basolateral amygdala (unpublished observations). Activity of GABAergic interneurons of the IC modulates the excitability of projection neurons in the medial geniculate body (MGB) of the thalamus via glutamatergic projections originating from the IC (Winer and Schreiner 2005; Peruzzi *et al*., 1997). These thalamic projections to the amygdala synapse on GABAergic interneurons in the lateral amygdala to modulate inhibitory tone in this structure (Woodson *et al*., 2000; Bauer and LeDoux 2004). Application of tetanizing stimulation to the MGB to augment thalamic output increases the amplitude of GABAergic inhibitory post synaptic potentials (IPSP) in the amygdala (Bauer and LeDoux, 2004). In this indirect manner, PCB-induced reductions in GAD concentrations and subsequent disinhibition of the IC may sensitize animals to AGS while at the same time augmenting afferent signaling to the MGB to increase amygdala inhibition and delay propagation of seizures from the kindling focus. Additional studies to interrogate the effects of PCB exposure on IC-MGB-basolateral amygdala circuitry are required to substantiate this hypothesis.

An alternative hypothesis is that the delay in amygdala kindling in PCB exposed rats stems from PCB-induced deficits in activity-dependent synaptic plasticity. Kindling and long term potentiation (LTP) are both models of synaptic plasticity. Kindling induces a potentiation similar in phenotype but larger in magnitude than that seen with LTP, and both kindling-induced potentiation and LTP are blocked by NMDA antagonists (Abraham and Mason, 1988; Gilbert and Mack, 1990). Unlike LTP, kindling requires the elicitation of an electrographic event, an AD, in order to occur and progress. LTP is transient, while kindling represents a permanent alteration in transsynaptic functionality (reviewed by Gilbert, 2001).

Consistent with delayed kindling development, PCB exposure has been shown to impair LTP. Acute perfusion of the single PCB congener PCB 77 (a dioxin like PCB congener) in mouse hippocampal slices led to reduced LTP and an increased threshold for the induction of LTP in area CA1 (Ozcan *et al*., 2004). It has also been shown that non-dioxin like PCB congeners (PCB153) can reduce LTP in area CA1 in rats (Hussain *et al*., 2000). Bath application of the non-dioxin congener (PCB153) or the complex PCB mixture, Aroclor 1254, reduced hippocampal LTP (Hussain et al., 2000; Niemi et al., 1998; Gilbert and Liang, 1998),). More relevant to the current study, developmental exposure to PCB 153 (Hussain et al., 2000) or PCB mixtures decreased LTP in CA1 of hippocampus (Curran et al., 2011) and in visual cortex slices from PCB77 exposed rats and mice (Altmann et al., 1998) . Similarly, LTP assessed in vivo in the dentate gyrus of the hippocampus was also impaired in adult rats following developmental exposure to the PCB A1254 mixture (Gilbert and Crofton, 1999; Gilbert *et al*., 2000). Collectively, these studies demonstrate direct compromise of synaptic function with acute PCB exposure to neuronal tissue as well as persistent reductions in activity-dependent synaptic plasticity following developmental exposure. A reduced capacity for use-dependent plasticity evidenced by LTP impairments as a result of developmental PCB exposure may contribute to the delay in electrical kindling observed in the present study.

The mechanisms whereby PCBs interfere with nervous system development to permanently alter synaptic function and plasticity remain elusive. Limited research indicates developmental exposure to PCBs can alter synaptic connectivity in the brain. In rodents, PCB exposure initially (at weaning) attenuates but later accelerates dendritic arborization of hippocampal neurons in rats exposed to Aroclor 1254 during times of central nervous system development (Lein *et al*., 2007) via a ryanodine receptor mediated calcium dysregulation (Wayman *et al*., 2012). Although the effects of developmental PCB exposure on neuronal dynamics have yet to be studied in the amygdala, it is possible they are similar to those seen in the hippocampus. In this manner, PCB-induced disruption of the degree and timing of neuronal growth in the developing nervous system may impair functionality, connectivity, and

plasticity critical for neuronal network formation critical for appropriate neuronal circuitry that supports kindling-induced plasticity in the adult.

5. CONCLUSIONS

In summary, in contrast to our previous findings of an enhanced susceptibility to AGS in the IC (Poon *et al*., 2015; Bandara *et al*., 2016), the present findings demonstrate a delay in electrical kindling of the amygdala in developmentally PCB exposed animals. Focal AD recorded from the amygdala was not affected by PCB exposure, but the behavioral manifestations of the kindling process were delayed. These results indicate an alteration in the cellular and physiological processes that underlie activity-dependent plasticity that drives this epileptogenic process. Developmental PCB-induced reductions in the capacity for activity-dependent plasticity revealed in this study or altered inhibitory signaling from the IC to the amygdala may underlie this observed retardation in kindling development. Although additional work is required to elucidate the mechanism of delayed kindling development, disruption in the functionality of the neurological substrates that support synaptic plasticity and altered network circuitry may contribute to these deficits and impairments in cognitive function associated with developmental PCB exposure in humans and animals.

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Figures

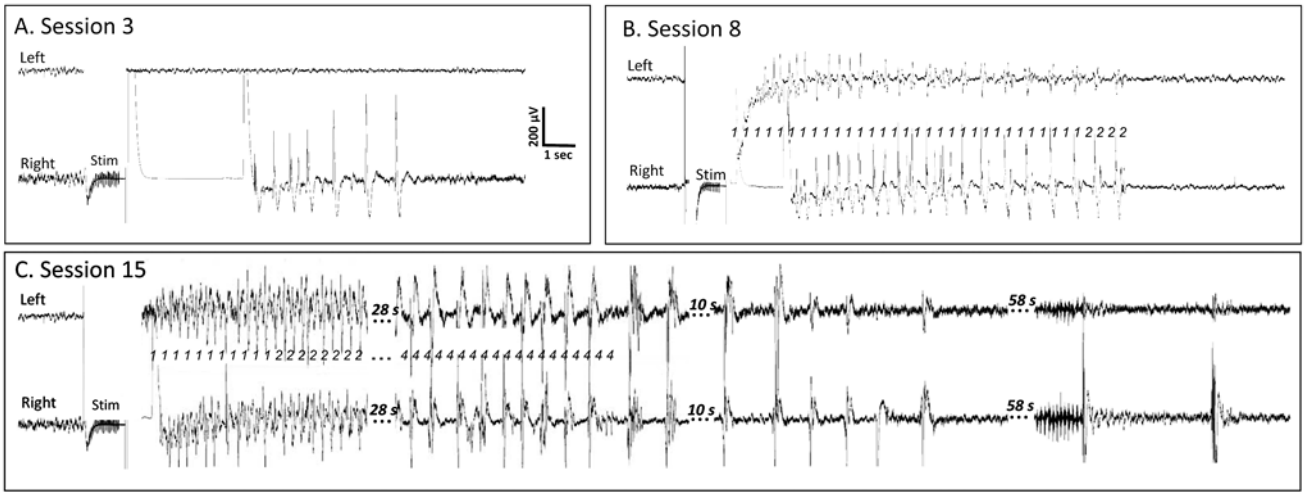


Figure 1. Electrographic representation of the development of kindling. Initially, A) short afterdischarge (AD) that outlasts the stimulation is limited to the stimulated site (Right) in early sessions with no apparent behavioral response. With daily repetition, B) AD evoked by the same stimulation lengthens and contralateral AD emerges, accompanied by behavioral signs of focal seizure activity. In later sessions as kindling progresses, C) primary AD increases in duration and complexity, secondary AD erupts after a period of electrical silence, and post ictal discharges are evident. The behavioral stages (1-4) that are observed during these ADs are indicated between the traces for right and left hemispheres.

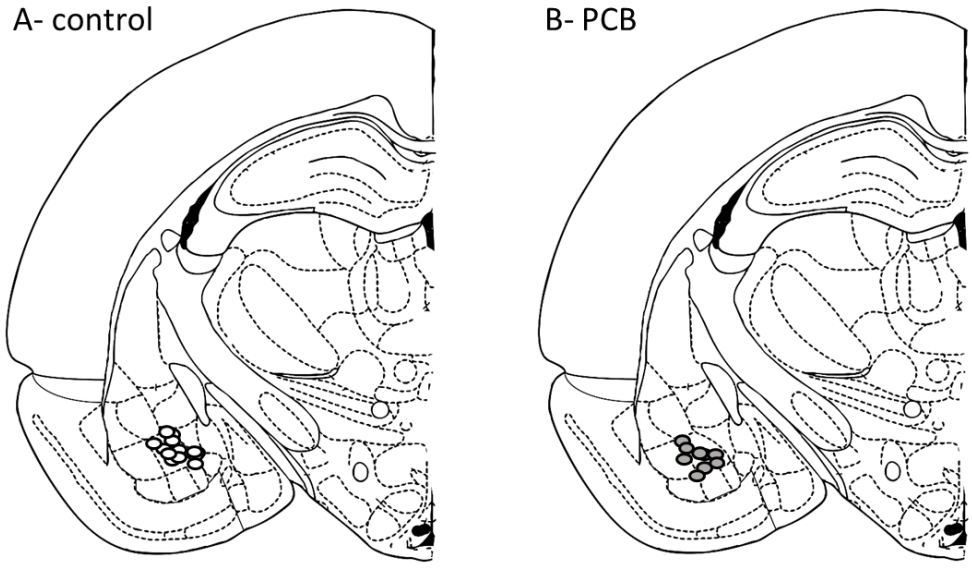


Figure 2. **Representative composite map of electrode placement for control and PCB exposed animals**. Modified image from Paxinos Watson 2006 Bregma -3.14 mm. A- White (control) and B- gray (PCB) circles represent the location of electrode tip placement for each animal. N= 12 for controls (representing 9 litters) and n= 9 for PCBs (representing 8 litters).

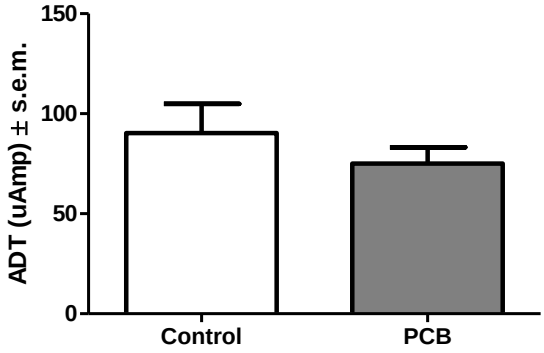


Figure 3. **Average after discharge threshold between PCB and control animals.** No significant effect of PCB exposure was seen. N= 8 and n= 9 litters for PCB and control groups were used respectively.

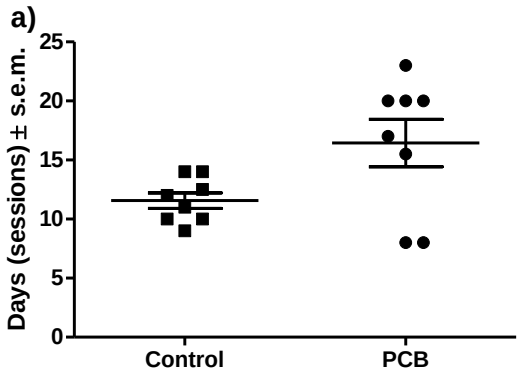
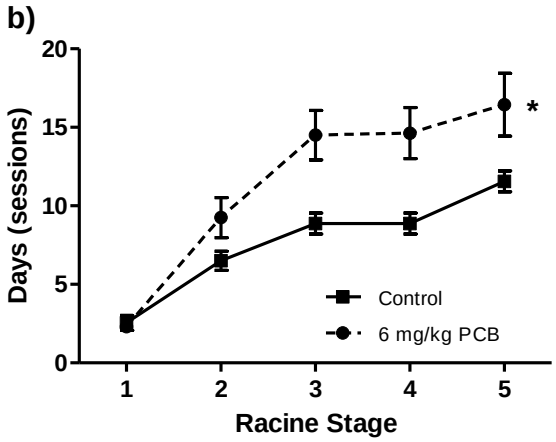
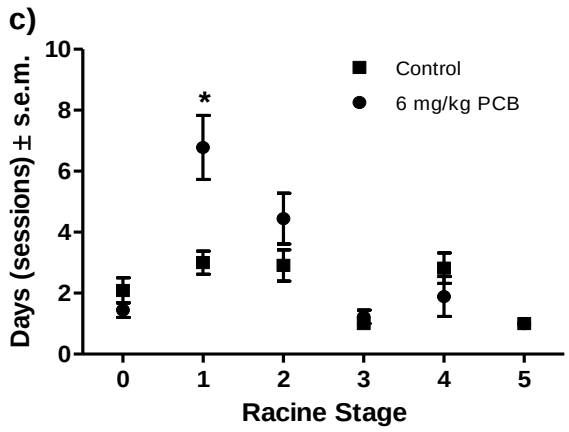


Figure 4. **a) Days to reach first Racine stage 5 seizure.** There was a significant effect where PCB exposed group (indicated in circles) took significantly longer compared to the control group (indicated in squares) ($p<0.05$).



b) Days to reach each Racine stage. * Indicates that there was a main effect where the 6 mg/kg PCB dose groups significantly differed from the control group ($p<0.05$).



c) Days in each Racine stage. The delay in kindling is mainly driven by the significantly longer duration (sessions) in stage 1 that the 6 mg/kg PCB animals took compared to the controls.

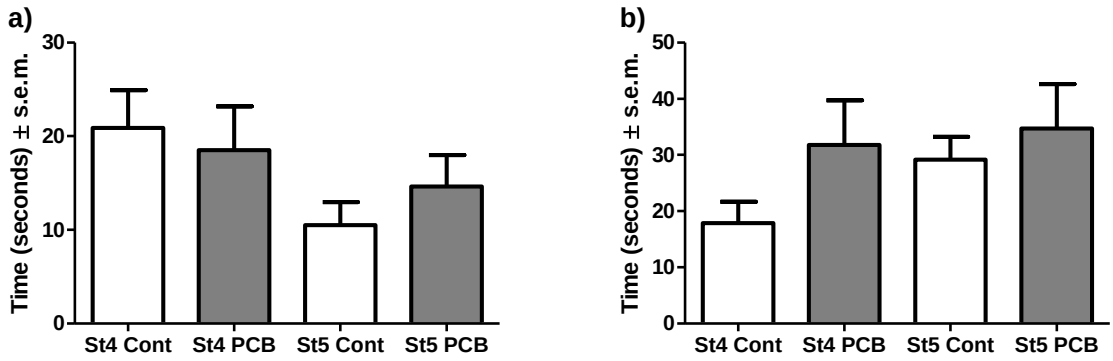


Figure 5. **Latency and duration of generalized seizures.** a) Latency to Racine stage 4 and stage 5. No significant effect of treatment was seen between the control and the PCB dose group. Similarly, b) the duration of clonus at Racine stage 4 and stage 5 also did not differ between the dose groups.

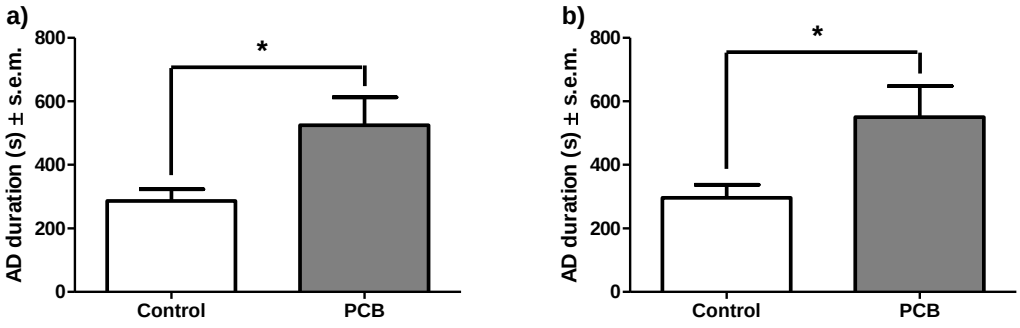


Figure 6. **Cumulative afterdischarge (AD) duration.** a) Cumulative primary AD duration and b) Cumulative total AD (primary + secondary/tertiary) AD duration for control and PCB exposed animals to reach the first stage 5 seizure. * Indicates that there was a significant effect where PCB exposed animals had longer ADs compared to control ($p<0.05$).

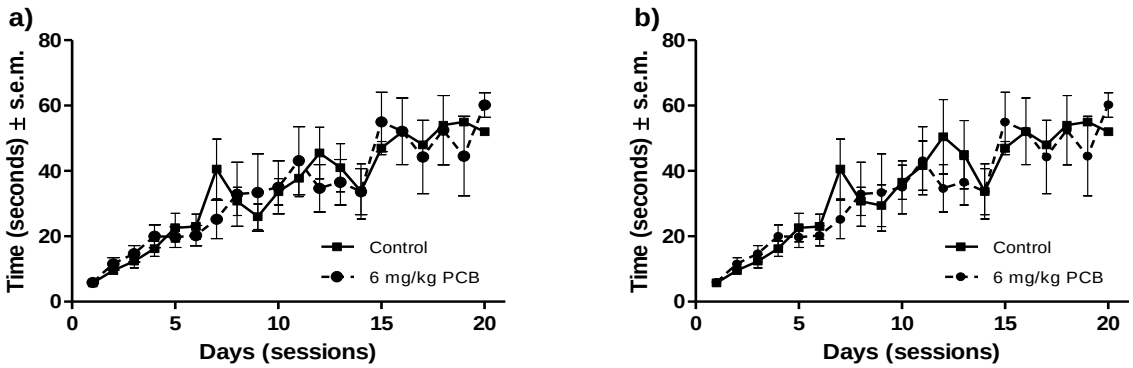


Figure 7. **Afterdischarge (AD) duration by session**. a) Primary AD duration by session and b) Total AD duration (primary+ secondary/tertiary) by session to the first stage 5 seizure does not differ between the control and the PCB dose groups.