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Journal: Toxicological Sciences
Manuscript ID: TOXSCI-13-0417.R1
Manuscript Type: Research Article
Date Submitted by the Author: 28-Jul-2013
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Key Words: polychlorinated biphenyls < Agents, neurotransmitter < Neurotoxicology, neurotoxicity; developmental < Neurotoxicology
Society of Toxicology Specialty Section Subject Area: Neurotoxicology [122]
Stimulation-Evoked Dopamine Release in the Nucleus Accumbens Following Cocaine Administration in Rats Perinatally Exposed to Polychlorinated Biphenyls

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Abstract

Exposure to polychlorinated biphenyls (PCBs) alters brain dopamine (DA) concentrations and DA receptor/transporter function suggesting the reinforcing properties of drugs of abuse acting on the DA system may be affected by PCB exposure. Female Long-Evans rats were orally exposed to 0, 3 or 6 mg/kg/day PCBs from 4 weeks prior to breeding until litters were weaned on postnatal day 21. In vivo fixed potential amperometry (FPA) was used in adult anesthetized offspring to determine if perinatal PCB exposure altered (a) presynaptic DA autoreceptor (DAR) sensitivity, (b) electrically evoked nucleus accumbens (NAc) DA efflux following administration of cocaine, and (c) the rate of depletion of presynaptic DA stores. One adult male and female littermate were tested using FPA following a single injection of cocaine (20 mg/kg IP), while a second adult male and female littermate were tested following the last of seven daily cocaine injections of the same dose. The carbon fiber recording microelectrode was positioned in the NAc core and DA oxidation currents (i.e., DA release) evoked by brief stimulation of the medial forebrain bundle (MFB) were quantified before and after administration of cocaine. PCB-exposed rats exhibited enhanced stimulation-evoked DA release (relative to baseline) following a single injection of cocaine. While non-exposed controls exhibited typical DA sensitization following repeated cocaine administration, this effect was attenuated in PCB-exposed rats. In addition, DAR sensitivity was higher (males only) and the rate of depletion of presynaptic DA stores was greater in PCB-exposed animals relative to non-exposed controls. These results indicate perinatal PCB exposure can modify DA synaptic transmission in the NAc in a manner previously shown to alter the reinforcing properties of cocaine.

Keywords: neurotoxicology, behavioral sensitization
Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental toxins that were banned in the late 1970s, but remain one of the most frequently identified contaminants in human adipose tissue, serum, and breastmilk (Safe, 1993). Ingestion of PCB-contaminated food is the main source of PCB exposure in human populations, while breathing contaminated air represents another minor exposure source (ATSDR, 2000). Exposure to mixtures of mostly non-coplanar PCB congeners typically represents the bulk of human exposure (Hansen, 1999).

The neurotoxic effects of non-coplanar PCBs may be explained by their ability to alter dopamine (DA) levels in the brain. Adult exposure to commercial Aroclor mixtures consisting of mostly non-coplanar PCB congeners has been shown to decrease DA concentrations in the striatum of rats (Seegal et al., 2002), mice (Richardson and Miller, 2004), and non-human primates (Seegal, 1996; Seegal et al., 1990; 1991; 1994). Perinatal exposure to Aroclor 1016 increased DA levels in several brain regions including the substantia nigra, caudate nucleus, and nucleus accumbens (NAc) in rats (Seegal, 1994), while exposure to the individual non-coplanar, ortho-substituted congener PCB 47 caused a significant decrease in DA concentration in rat caudate nucleus and frontal cortex (Seegal et al., 1997).

Non-coplanar PCBs inhibit the DA transporter (DAT; Mariussen and Fonnum, 2001), leading to increased DA in the synapse. This finding may appear to contradict research demonstrating that PCBs decrease extracellular DA levels, but Seegal et al. (2002) reported that PCB-induced elevations in extracellular DA levels were transient. Basal extracellular DA levels were elevated in adult rat striatum following three days of exposure to Aroclor 1254, but after one week of exposure, levels were significantly decreased. One explanation for this biphasic response may be because non-coplanar PCBs also inhibit the activity of the vesicular monoamine
transmitter (VMAT2) which transports monoamines like DA into vesicles in the presynaptic terminal for later release (Mariussen et al., 1999). The increase in cytosolic DA may lead to end-product inhibition of tyrosine hydroxylase (TH), the rate-limiting enzyme of intraneuronal DA synthesis (Cooper et al., 1996). Bemis and Seegal (2004) demonstrated that PCB inhibition of VMAT2 seemed to play a greater role than the inhibition of DAT on decreasing tissue DA. They observed a weak (non-significant) relationship between PCB-induced elevations in media DA and reductions in synaptosomal DA. Known DAT inhibitors elevated media DA significantly more than PCBs while having similar effects on tissue DA content. Known VMAT inhibitors, however, elevated 3,4-dihydroxyphenylacetic acid (DOPAC; the intraneuronal metabolite of DA) and decreased tissue DA to the same degree that PCBs did. Fewer DATs and VMATs are expressed within the striatum of PCB-exposed adult laboratory animals (Caudle et al., 2006; Richardson and Miller, 2004) and in synaptosomes during *in vitro* exposure (Fonnum et al., 2006).

There is also evidence to suggest that DA autoreceptor (DAR) activity can influence DAT and VMAT2 function, but the effects of PCB exposure on DAR sensitivity have not been investigated. With respect to DAT, in vitro and in vivo evidence has suggested that pharmacological blockade of DAR activity or reduction in DAR expression results in a decrease in DAT efficiency. Increased extracellular DA levels that accompany reductions in DAR activity lead to a compensatory increase in DAT velocity, but this is insufficient to overcome the reduction in DAT efficiency (Dickinson et al., 1999; Zahniser et al., 1999; Mayfield et al., 2001; Wu et al., 2002). Thus, stimulation of DARs increased DAT function, whereas blockade of DARs by DA D2 antagonists decreased DAT function, and DAR sensitivity has been shown to be positively coupled to DAT function (Gulley & Zahniser, 2003). Likewise for VMAT2, it has
been shown that increased extracellular DA caused by the uptake blockers cocaine and methylphenidate cause the redistribution of VMAT2-containing vesicles from a plasmalemmal membrane fraction to a presumably cytosolic fraction. This cocaine-induced vesicular trafficking is blocked with DA D2 receptor antagonists, suggesting a potential involvement with presynaptic D2 DARs. Moreover, administration of D2 agonists has been shown to cause vesicle trafficking in a manner similar to uptake blockers, suggesting that increased extracellular concentrations of DA in response to DAT blockade may stimulate DARs causing vesicles to move into the cytosol, thus facilitating release (see Riddle et al., 2005).

Because DA levels within the mesolimbic dopaminergic pathway influence psychostimulant reinforcement and addiction (Pierce and Kumaresan, 2006), the relationship between drug addiction and PCB exposure warrants further investigation. Animals that have been exposed to PCBs exhibit behaviors that suggest that they may have a higher risk of developing drug addiction (Jones and Miller, 2008). For example, deficits on inhibitory control tasks in rats (Holene et al., 1998; 1999; Sable et al., 2006; 2009), monkeys (Mele et al., 1986; Rice, 1997; 1998), and humans (Jacobson et al., 1992; Stewart et al., 2003; 2005; 2006) perinatally exposed to PCBs have been demonstrated. Likewise, interoceptive cues following administration of cocaine and amphetamine (Sable et al., 2011), as well as locomotor activation and behavioral sensitization following amphetamine administration (Poon et al., 2013) have all been reported to be altered in rats perinatally exposed to PCBs.

The aim of the present study was to examine the relationship between developmental exposure to PCBs and mesolimbic DA functioning associated with psychostimulant administration. In vivo fixed potential amperometry (FPA) was used in anesthetized adult offspring to determine if perinatal PCB exposure altered (a) presynaptic DA autoreceptor (DAR)
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sensitivity, (b) electrically evoked nucleus accumbens (NAc) DA release following administration of cocaine, and (c) the rate of depletion of presynaptic DA stores. The hypotheses were that relative to non-PCB exposed rats, those perinatally exposed to PCBs would exhibit (a) enhanced DAR sensitivity, (b) elevated levels of stimulated DA release in the NAc (relative to baseline) following cocaine administration, and (c) greater stimulation-induced depletion of DA vesicular stores. These predictions were made based on the ability of PCBs to (a) decrease basal DA levels thereby resulting in greater DAR sensitivity, (b) decrease DAT expression thereby causing greater DAT occupancy by cocaine, and (c) decrease VMAT2 expression thereby resulting in decreased sequestration of DA into synaptic vesicles for later release.

Materials and Methods

Animals and PCB Exposure

Nulliparous female Long Evans rats obtained from Harlan Laboratories (Indianapolis, IL) were individually housed in standard plastic cages and maintained on a 12 hour light/dark cycle with free access to food (Harlan Teklad 2020X) and water. Beginning 28 days prior to breeding (i.e., approximately 49 days before birth) and continuing until pups were weaned on PND 21, these females were given oral doses of an environmentally relevant PCB mixture. The mixture was formulated to mimic the congener profile that is observed in walleye taken from the Fox River in northeast Wisconsin and consisted of 35% Aroclor 1242, 35% Aroclor 1248, 15% Aroclor 1254, and 15% Aroclor 1260. The congener profile of this mixture is provided in detail elsewhere (see Kostyniak et al., 2005) along with the aryl hydrocarbon receptor and ryanodine receptor activity of the mixture (which was determined to be relatively low and high, respectively).
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The dose groups were balanced for body weight and consisted of either 3 or 6 mg/kg/day of the PCB mixture (dissolved in corn oil) or 0 mg/kg/day PCBs (i.e., corn oil only; n=8 dams/exposure group). These doses were chosen because offspring born to dams at these doses experience weight reductions at birth and weaning (Kostyniak, et al., 2005) and cognitive deficits (Sable and Schantz, 2006) similar to those reported in humans exposed to PCBs (Fein et al., 1984; Jacobson and Jacobson, 2003; Jacobson et al., 1990; Stewart et al., 2006). Dams were weighed daily and the volume of administered dosing solution adjusted to account for weight gain. A positive displacement pipette was used to deliver the correct volume of the PCB dosing solution (or corn oil for the controls) onto one-half of a small vanilla cookie (Golden Vanilla Wafers, Keebler®) which was fed to the dam. The dams generally consumed their cookie in 15 min or less and consumption of the cookie was visually confirmed each day.

At parturition (PND 0), pups were counted, weighed, sexed, and any abnormalities were noted. Large litters were culled to 10 pups on PND 2 (5 males, 5 females, when possible) and the extra pups cross-fostered (if needed) to litters in the same exposure group that had at least 7 pups. The cross-fostered pups were not used in FPA testing; they served to ensure that pups to be tested did not receive a disproportionate amount of PCB exposure (via lactation from exposed dams). When the pups became mobile, the dam was removed and placed in a separate cage for dosing to ensure the pups were not directly eating the dosed cookie. On PND 21, pups were weaned from the dams, and two females and two males from each litter were kept for FPA testing (n = 16 males and 16 females/exposure group) which was performed after the offspring reached adulthood (~PND 60). See Figure 1 for an experimental timeline.

Gestational, lactational, and postnatal outcomes using this dosing paradigm have been reported previously (see Kostyniak et al., 2005; Sable et al., 2006; 2009; 2011). All procedures
were approved by the University of Memphis Institutional Animal Care and Use Committee and were in accordance with the guidelines of the Public Health Service Policy on Humane Care and Use of Laboratory Animals (National Institutes of Health, 2002) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003).

**Apparatus**

DA release in the NAc was recorded *in vivo* using a commercially available hardwired electrochemical recording system (eDAQ, Pty Ltd.) consisting of a potentiostat (EA161 Picostat) in conjunction with an analog-to-digital signal converter-recorder (e-corder). For every molecule of dopamine that was oxidized (as a result of release), two electrons were transferred to the recording electrode. An A/D converter converted the changes in DA oxidation current to a digital voltage signal that allowed the observation of stimulation-evoked changes in DA release in real-time.

**Cocaine Administration**

One male and female per litter were randomly selected and placed in the single cocaine injection group (n=8 males and 8 females/exposure group) while the other male and female retained were placed in the repeated cocaine injection group (n=8 males and 8 females/exposure group). The rats in the single injection group received an intraperitoneal (IP) injection of 20 mg/kg cocaine on the same day of FPA testing, while rats in the repeated injection group received IP injections of 20 mg/kg cocaine on each of the six consecutive days prior to FPA testing and again on the same day as FPA testing (seven total daily injections).

**FPA Testing**
Rats were anesthetized with a 1.5 mg/kg IP of urethane. If needed, supplemental doses were administered until rats did not exhibit a pain response (determined via tail pinch). Anesthetized rats were placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) located within a Faraday cage. After leveling of the skull so that lambda and bregma were on the same horizontal plane, the cranium was exposed and three bur holes were drilled unilaterally (two holes) and contralaterally (one hole) using a dental drill. Coordinates for the placement of electrodes within the MFB and NAc core were obtained using the rat atlas of Paxinos and Watson (1998). A bipolar stimulating electrode (SNE-100; Rhodes Medical Co.) was placed into the medial forebrain bundle (MFB) at the following coordinates: AP:-4.2 mm from bregma, ML: 1.8 mm, and DV:-7.8 mm from dura mater. A carbon fiber recording electrode (carbon fiber 10um outer diameter, 250um length, Thornel Type P, Union Carbide, PA) was placed in the NAc core at the coordinates: AP: + 1.6 mm from bregma, ML: 1.5 mm, DV: -7.4 mm from dura mater. An Ag/AgCl reference/auxiliary combination electrode was placed contralateral to the stimulating and recording electrodes on the surface of the cortex. The auxiliary electrode applied a fixed electrical potential of +0.8 V to induce the oxidation of DA at the carbon fiber electrode. As DA was released within the NAc, it was oxidized on the surface of the recording electrode. Thus, it was the change in electrical oxidation current that permitted for the measurement of DA release.

Fixed potential amperometry coupled with carbon fiber microelectrodes has been confirmed as a valid technique for real-time monitoring of DA oxidation current evoked by electrical stimulation of ascending mesoaccumbens dopaminergic projections (Benoit-Marand et al., 2000; Dugast et al., 1994; Lester et al., 2008; Schönfuß et al., 2001; Suaud-Chagny 2004). Confirmation of the chemical specificity of our amperometric measurements has been provided.
by previous studies (e.g., Dommett et al., 2005; Lee et al., 2006; Mittleman et al., 2008), showing that systemic administration of the selective DA reuptake inhibitor nomifensine selectively increases electrical stimulation-evoked DA oxidation current and delays recovery to prestimulation baseline levels.

Baseline DA release

Baseline recordings of DA release (upon stimulation of the MFB) were recorded for two minutes for all rats. Fifteen cathodic monophasic pulses at 50Hz (1000 µA intensity, 0.5 ms pulse duration) were applied to the stimulating MFB electrode every 30 seconds and DA release determined as described above. The measurement of stimulation-evoked DA efflux during baseline was quantified during the last 30 seconds of baseline by determining the peak height of the electrical oxidation current.

DAR sensitivity

The procedure to measure DAR sensitivity using FPA has been validated by Benoit-Marand et al. (2000). This study used essentially the same procedure. Briefly, DAR sensitivity for each rat was determined by applying a pair of test stimuli (T1 and T2; stimulation parameters the same as during baseline) to the MFB every 30s to evoke DA release. Five sets of conditioning pulses (5, 10, 20, 40, and 80; 0.5-ms pulse duration at 15 Hz) were delivered prior to T2 such that there was 0.3s between the end of the conditioning pulse train and initiation of T2. The effects of the conditioning pulses on T2 stimulated DA efflux relative to T1 (which served as baseline) were measured in ascending order (i.e., the effects of 5 conditioning pulses was determined first, followed by 10 conditioning pulses, followed by 20, etc.). T1 preceded the T2 stimulus by approximately 10 seconds. Between trials the next T1 stimulus occurred approximately 20 seconds after the previous T2 stimulus and was recorded in the absence of any...
conditioning pulses thereby serving as a moving baseline response (100%). This allowed for the
determination of the effect of each conditioning pulse train on DAR for T2. For each set of
conditioning pulses, four T1-T2 trials were administered. DAR-mediated inhibition of evoked
DA release was expressed in terms of the change in the amplitude (i.e., peak height) of T2 with
respect to T1 (T2/T1*100) for each using the third trial within each set of conditioning pulses
such that low-to-high DAR sensitivity represented low-to-high percent inhibition of evoked DA
release (i.e., high sensitivity resulted in a lower amplitude of T2 relative to T1; see Supplemental
Figure 1). The entire sequence took approximately 10 min to complete.

DA release following IP cocaine

After baseline recordings, the effect of cocaine on MFB stimulation-evoked DA release was
then measured for 60 min using the same stimulation parameters applied during baseline (pre-
cocaine). In the repeated injection group, rats received their seventh IP injection of 20 mg/kg of
cocaine and then MFB stimulation-evoked DA release was continuously recorded for an hour.
Rats in the single injection group received their first IP injection of cocaine (20mg/kg) following
baseline, and their DA release was also recorded for an hour. The percent change from baseline
MFB stimulation-evoked DA release and the time point of peak DA release (again measured as
peak height) following the injection of cocaine were determined for each rat. See Supplemental
Figure 2.

Determination of pre-synaptic DA stores

For this last measure, stimulation- evoked DA release was recorded during continuous (5
min) MFB stimulation (50Hz, 1000 µA intensity, 0.5 ms pulse duration). Since amperometry
measures phasic DA levels by recording the number of DA molecules that are released
(oxidized), it is a better indicator of how much DA is being released rather than how much is
present at a specified time. Consequently, oxidation current values indicate the amount of DA that is being released and, thus, the ease of depletion of MFB stimulation-evoked DA release. Since oxidation current is a reflection of DA release, higher values indicate relatively larger vesicular pools of DA available for release upon electrical stimulation of the MFB. The sum of the oxidation current (nAmps) across the period beginning 30 seconds and ending 90 seconds after the start of continuous stimulation was used.

**Stereotaxic Placement Verification**

After amperometry testing was completed, direct anodic current (100 mA for 10 s) was applied to the stimulating electrode in the MFB (AP:-4.2 mm from bregma, ML: 1.8 mm from bregma, and DV:-7.8 mm from dura mater.) and NAc core (1.6mm from bregma, ML: 1.5 mm from bregma, DV: -7.4 mm from dura mater). Sedated rats were then euthanized via decapitation, and their brains were removed and prepared in 30%/10% sucrose/formalin and 0.1% potassium ferricyanide for cryostat sectioning. Potassium ferricyanide stained the lesioned sites of the recording and stimulating electrodes, such that correct electrode positioning could be verified. All rats were determined to have correct placements of both electrodes.

**Data Analysis**

All data was analyzed using SPSS software (version 21). Peak DA release (relative to baseline), the time point of peak DA release following the cocaine injection, and the aforementioned oxidation current following continuous MFB stimulation were separately analyzed via a 3 (exposure group) x 2 (injection history) x 2 (sex) mixed ANOVA. Exposure group was a between-subjects factor while injection schedule (single vs. repeated) and sex were repeated-measures factors that were both nested within litter. (There were four animals from each litter with one male and one female placed in the single cocaine injection group and the
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other male and female placed in the repeated cocaine injection group.) DAR sensitivity was
analyzed similarly but also included number of conditioning pulses (5, 10, 20, 40, and 80) as an
additional repeated-measures factor. In the interest of brevity, only significant exposure-related
and sex-related main effects and interactions are reported. Additional post-hoc analyses were
conducted as appropriate to determine the nature of significant effects that were found in the
initial omnibus analyses.

Results

DAR Sensitivity

The omnibus analysis of the amplitude of T2 relative to T1 baseline (T2/T1*100)
revealed several significant effects. Significant main effects of PCB exposure \(F(2,21)=5.089,\
\(p=.016\), sex \(F(1,21)=29.871, p<.001\), and number of conditioning pulses \(F(4,84)=65.628,\
\(p<.001\) were found, as well as a significant PCB exposure x sex interaction \(F(2,21)=4.196,\
\(p=.029\). As expected, the amplitude of T2 relative to T1 decreased (indicating DAR sensitivity
increased) across all groups as the number of conditioning pulses increased, with the greatest
magnitude of attenuation being measured at 80 conditioning pulses (see Supplemental Figure 1).
Following this longest conditioning pulse train, females within the 0 and 6 mg/kg/day PCB
exposure groups exhibited significantly greater DAR sensitivity than the males (\(p=.019\) and .009,
respectively). Post hoc Dunnett analyses revealed that for the males but not the females, DAR
sensitivity increased in a PCB exposure-dependent manner (see Figure 2).

DA release following cocaine administration

Percent Change From Baseline

For each rat, peak DA release was recorded during baseline and again during the cocaine
challenge. The change in MFB-stimulated DA release relative to the pre-injection baseline was
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calculated for each rat and expressed as the percent changes with respect to the pre-injection baseline response (100%). The analysis revealed a significant PCB exposure group x injection schedule interaction \[ F(2,21)=10.757, p=.001 \] (Figure 3). Dunnett post hoc tests conducted on the data from rats receiving their first injection of cocaine revealed the 6mg/kg/day PCB group was significantly different from control group \( p=.001 \), while the difference between rats exposed to 3 mg/kg/day PCBs and controls only approached significance \( p=.055 \). For rats tested following repeated cocaine injections, there was only a significant difference between rats exposed to 3 mg/kg/day and control rats \( p=.019 \). Post hoc simple effects analyses revealed that within the control group, there was a significant difference between rats receiving single versus repeated cocaine injections \( p=.005 \) but there were no significant differences between rats receiving single versus repeated cocaine injections in the 3 or 6 mg/kg/day PCB groups (Figure 3).

The omnibus analysis also revealed a significant main effect of sex \[ F(1,21)=7.373, p=.013 \]. Females had a greater amount of DA release (expressed as percent change from pre-injection baseline) following cocaine administration compared to males (Mean ± SEM: 162.098 ± 8.676 and 166.170 ± 9.111 for males and females, respectively). No other significant main effects or interactions were found.

Time Course of Peak DA Release

The 30 second interval at which the electrical oxidation current was the highest for each rat was recorded as the peak of MFB-stimulated DA release and was recorded as min post injection. These data are presented in Table 1. The peak of stimulated DA release appeared to occur earlier in male rats that received repeated injections of cocaine following perinatal exposure to 3
mg/kg/day PCBs. However, no significant exposure-related or sex-related effects were found in
the omnibus analysis.

**Dopamine release during the continuous stimulation of the MFB**

The sum of oxidation current (nAmps) was measured in all three exposure groups
beginning 30 seconds and ending 90 seconds after the start of continuous stimulation and clearly
decreased with increasing PCB dose. While the main effect of exposure only approached
significance \[ F(2,21)=3.101, p=.066 \], a large effect size (Cohen, 1988) was found (partial
\( \eta^2 = .22 \); see Figure 4) justifying further post-hoc analyses. Dunnett post hoc testing revealed a
significant difference only between rats in the 6mg/kg/day exposure group and rats in the control
group \( (p=.047) \). There were no other significant effects.

**Discussion**

Several recent studies have reported perinatal PCB exposure alters the behavioral
response to psychostimulants (Poon et al., 2013; Sable et al., 2009; 2011) and numerous studies
have reported that exposure to polychlorinated biphenyls (PCBs) alters brain dopamine (DA)
concentrations (Richardson and Miller, 2004; Seegal, 1994; 1996; Seegal et al., 1990; 1991;
1994; 1997; 2002) and DA receptor/transporter function (Mariussen and Fonnum, 2001;
Mariussen et al., 1999). Thus, disruption in typical DA functioning caused by perinatal PCB
exposure was thought to be the mechanism responsible for the aforementioned behavioral
differences observed in PCB-exposed rats following administration of psychostimulants. This
study was conducted to more directly assess this potential mechanism. Specifically, measures of
DA autoreceptor sensitivity, differences in DA release following cocaine, and the rate of
depletion of presynaptic DA stores were examined in rats perinatally exposed to PCBs and in
non-exposed control rats.
The first hypothesis that animals exposed to PCBs would show greater DA autoreceptor sensitivity was supported, although the effect was sex-specific (see Figure 2). The conditioning pulses administered in the present study were intended to activate terminal autoreceptors prior to DA release caused by MFB stimulation at T2. As such, greater sensitivity in DARs would translate into greater attenuation of DA release at T2. DAR sensitivity increased overall for all groups as the number of conditioning pulses increased. In addition, male rats perinatally exposed to PCBs exhibited a smaller amplitude of T2 with respect to T1 (T2/T1*100) compared to non-exposed controls (i.e., PCB dose = 0 mg/kg/day) - an effect not observed in the females. Terminal autoreceptors are known to monitor synaptic DA (Wolf and Roth, 1987) and modulate both the synthesis (Kehr et al., 1972) and release (Farnebo and Hamberger, 1971) of DA. Given the ability of PCB exposure to alter basal DA levels in numerous brain areas including striatum (Richardson and Miller, 2004; Seegal, 1996; Seegal et al., 1990a; 1991; 1994; 2002), nucleus accumbens (Seegal, 1994), and frontal cortex (Seegal et al., 1997), the finding that these terminal DARs exhibited altered sensitivity was not unexpected. Interestingly, there was also a difference between the male and female control groups. It is unclear why this sex difference occurred and a search of the literature did not reveal any other studies that examined this issue in the rat strain used in this study (Long-Evans). Notably, gender differences in DAT and VMAT2 function have been reported in mice (Ji et al., 2009). Given the inter-relationship previously discussed between DATs, VMAT2s, and DARs, this sex difference in DAR sensitivity reported here (while not well understood) is not surprising and will require further examination.

The differential effect of perinatal PCB exposure on male versus female DAR sensitivity is in line with other studies in the PCB literature that have reported males perinatally exposed to PCBs to be more affected than similarly exposed females. For example, neurobehavioral studies
have reported male rats, but not females, perinatally exposed to PCBs exhibited changes in amphetamine behavioral sensitization (Poon et al., 2013) and decreased performance on operant tasks known to assess response inhibition and timing (Holene et al., 1998; 1999; Sable et al., 2006; 2009). In addition, male rats perinatally exposed to PCBs have been shown to exhibit feminization of sweet-preference behavior (i.e., sweet preference in PCB-exposed males approached that typically seen in non-exposed females), while sweet preference in the females (which was much higher overall) was not affected by PCB exposure (Hany et al., 1999; Kaya et al., 2002).

The influence of DARs as a feedback mechanism for DA synthesis (Kehr et al., 1972) and DA release (Farnebo and Hamberger, 1971) have also been well-documented. Using fast-scan cyclic voltammetry in dorsal striatal slices, a single-pulse procedure (across a range of stimulus intensities 150-600µA) produced a significantly higher amount of DA release in mice deficient in D2 autoreceptors (autoDrd2KO mice) compared to control mice (Bello et al., 2011). Likewise, when the D2 agonist quinpirole (0.5 mg/kg BW IP) was given prior to IP administration of the aromatic amino acid decarboxylase inhibitor NSD1015 (100 mg/kg IP), the decrease in L-DOPA activity seen in the control mice did not occur in the autoDrd2KO mice, suggesting a definitive role for the D2 receptor in regulating DA synthesis. In fact, L-DOPA accumulation following NSD1015 administration was the same in autoDrd2KO mice with and without quinpirole pre-treatment. These results suggest D2 receptors may be the only receptor subtype involved in the autoreceptor feedback inhibition of DA synthesis (Bello et al., 2011). Further support of this claim has been in found in studies using mice lacking the DA D3 receptor, where DA synthesis (as measured by DOPA accumulation) was the same in the mutant and wild-type mice after pre-treatment with the D3 agonist PD 128907 (Koeltzow et al., 1998). Overall,
these results suggest changes in the sensitivity of DARs (particularly the D2 subtype) that occur following PCB exposure would be expected to alter DA synthesis and release.

Coccini et al. (2011) reported that exposure to PCB 153 (a di-ortho, non-coplanar PCB congener) from gestational day 7 – PND 21 increased cortical (but not striatal) expression of D2 receptor density at weaning (PND 21) in the male, but not female, weanlings. By PND 36, cortical D2 receptor density was elevated in both the PCB-exposed males and females. D2 receptor affinity was reduced in cortex for both sexes at both time points. While Coccini et al. (2011) did not specifically examine the effects of PCB exposure on D2 autoreceptor expression/affinity, future research is needed examine this possibility.

The second hypothesis that PCB-exposed rats would exhibit elevated levels of stimulated DA release in the NAc (relative to baseline) following administration of cocaine was also supported, but only in the rats receiving their first injection of cocaine. Following the first injection of cocaine, there was a dose-dependent effect as DA release increased as PCB exposure increased. This was not the case, in the rats that had received prior daily injections of cocaine. With repeated cocaine injections, electrically stimulated DA release in non-exposed controls increased as is typical of psychostimulant sensitization. In rats perinatally exposed to PCBs, however, peak evoked DA release following repeated cocaine injections was attenuated (see Figure 3). These results are in line with a previous study examining the effects of developmental PCB exposure on the behavioral response to psychostimulants. Poon et al. (2013) observed that when given a single dose of amphetamine, male (but not female) rats perinatally exposed to PCBs showed increased locomotor activation (relative to controls). After repeated amphetamine injections, the PCB-exposed males showed attenuated locomotor sensitization rather than typical amphetamine-induced behavioral sensitization as was seen by the non-exposed control group.
The results of the current study suggest altered DA release within the NAc (possibly tied to increased DAR sensitivity and greater depletion of presynaptic DA stores) in PCB-exposed animals are one mechanism responsible for differences in the behavioral activating effects of amphetamine and amphetamine behavioral sensitization in these animals.

The final hypothesis of this study was also supported as the measurement of oxidation current in the NAc core upon the continuous stimulation of the MFB indicated that releasable DA stores are relatively reduced in PCB-exposed rats (see Figure 4). Recall that since oxidation current is a reflection of DA release, higher values indicate relatively larger vesicular pools of DA available for release upon electrical stimulation of the MFB. DA stores were depleted faster in PCB-exposed rats, with a significant difference between the control group and highest exposure group.

Previous studies suggest that PCB-induced VMAT2 inhibition is mainly responsible for decreased DA levels (Bemis and Seegal, 2004). However, the ability of PCBs to alter DAR sensitivity, to differentially alter stimulated DA release after single versus repeated cocaine exposure, and to reduce presynaptic DA stores also suggests that PCB-induced DAT inhibition and increased DAR sensitivity may contribute to the observed changes in DA levels frequently reported following PCB exposure (Richardson and Miller, 2004; Seegal, 1994; 1996; Seegal et al., 1990; 1991; 1994; 1997; 2002). Evidence for the importance of DAT function on DA synthesis, storage, and release was provided by Jones et al. (1998) who used DAT genetic mutant mice and determined that wildtype (DAT +/+ ) mice had the highest amount of striatal DA release compared to heterozygote (DAT -/+ ) and homozygote (DAT -/- ) mice. Dramatic reductions in presynaptic DA stores and release (95 % and 75%, respectively) as well as decreased tyrosine hydroxylase (TH) levels were observed in the DAT -/- mice. Like these mice,
rats exposed to PCBs have been shown to have lower DAT expression (Lyng et al., 2007; Richardson and Miller, 2004) which likely contributes to the reduction in presynaptic DA stores and stimulated DA release found in this study.

The present findings have several implications for humans who have been exposed to PCBs, and/or other neurotoxicants that affect DA function in the brain. Cocaine-induced increases in extracellular DA within the NAc contribute to the reinforcing properties of cocaine (Carelli, 2002; Koob et al., 1994), leading to sensitized dopamine systems and enhanced incentive salience in addicts for drugs of abuse. Enhanced incentive salience results in an increased sense of “wanting” in the human population (Berridge et al., 2009; Robinson and Berridge, 2008).

PCB exposure resulted in increased MFB-stimulated DA release following the first injection of cocaine. This enhanced efficacy for cocaine in PCB-exposed individuals could be expected to produce elevated levels of reward which could promote enhanced drug-seeking. In addition, a decrease in stimulated DA release occurred following daily repeated injections of cocaine which could be tied to escalation of cocaine use in PCB-exposed individuals. To address this issue, research is currently underway which is examining acquisition and maintenance of cocaine intravenous administration in rats perinatally exposed to PCBs.

In conclusion, PCBs affect DA regulation on several levels- including DA synthesis, DA reuptake and storage, and DA release (Angus et al., 1997; Bemis and Seegal, 2004; Mariussen and Fonnum, 2001; Seegal et al., 1989; 1990; 1997; 2002). The results of this study provide important evidence that perinatal PCB exposure has the ability to alter the typical neurochemical response to psychostimulants. These results therefore suggest that perinatal PCB exposure may modulate behaviors related to drug reward and/or drug-seeking.
Funding

This work was supported by the National Institute of Environmental Health Sciences at the National Institutes of Health [grant number R00ES015428].

Acknowledgements

The authors would like to thank Dr. Larry Hansen for his help in formulating the PCB mixture and Dr. Karyl Buddington for her outstanding veterinary support.
COC-INDUCED DA RELEASE IN PCB-EXPOSED RATS

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(PCA153) affects cerebral dopamine D1-like and D2-like receptors of weanling and pubertal rats. *Arch Toxicol* 85, 1281-94.


COC-INDUCED DA RELEASE IN PCB-EXPOSED RATS


TABLE 1

Time of Peak MFB-Stimulated Dopamine Release in the Nucleus Accumbens Core (Min Post Injection) Following 20.0 mg/kg Cocaine (IP) For Each PCB Exposure Group

<table>
<thead>
<tr>
<th>PCB Exposure Group (mg/kg/day)</th>
<th>Single Injection Group</th>
<th>Repeated Injection Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control; 8 litters)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVERALL</td>
<td>17.86 ± 3.09</td>
<td>21.56 ± 4.86</td>
</tr>
<tr>
<td>MALE</td>
<td>20.00 ± 6.05</td>
<td>25.63 ± 7.47</td>
</tr>
<tr>
<td>FEMALE</td>
<td>15.71 ± 5.38</td>
<td>17.50 ± 5.57</td>
</tr>
<tr>
<td>3 (8 litters)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVERALL</td>
<td>18.79 ± 3.73</td>
<td>9.73 ± 2.82</td>
</tr>
<tr>
<td>MALE</td>
<td>26.88 ± 6.61</td>
<td>3.75 ± 2.63</td>
</tr>
<tr>
<td>FEMALE</td>
<td>10.71 ± 3.19</td>
<td>15.71 ± 3.83</td>
</tr>
<tr>
<td>6 (8 litters)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVERALL</td>
<td>14.11 ± 2.92</td>
<td>15.67 ± 3.11</td>
</tr>
<tr>
<td>MALE</td>
<td>15.71 ± 5.54</td>
<td>10.71 ± 3.46</td>
</tr>
<tr>
<td>FEMALE</td>
<td>12.50 ± 2.50</td>
<td>20.63 ± 5.46</td>
</tr>
</tbody>
</table>

Note. Means (± SEM). MFB = medial forebrain bundle; IP = intraperitoneal; PCB = Polychlorinated biphenyls
Figure 1. Experimental timeline denoting duration of PCB exposure, weaning, onset of daily cocaine intraperitoneal (IP) injections, and fixed potential amperometry (FPA) testing. 1 0, 3, or 6 mg/kg/day PCBs (n=8 dams/PCB exposure group); 2 two male and two female offspring retained/litter (n=16 males and 16 females/PCB exposure group); 3 20 mg/kg (IP) cocaine given to one male and one female offspring/litter (n=8 males and 8 females/PCB exposure group); 4 second male and second female from each litter did not receive repeated daily injections of cocaine (n=8 males and 8 females/PCB exposure group)); 5 DA release measured during baseline, during assessment of DAR sensitivity, in response to 20 mg/kg cocaine (IP), and during continuous stimulation in all offspring. Figure 1 is a timeline showing the experimental timeline with the following details:

- **PCB Exposure**:
  - Dosing Begins: PND -49
  - Gestation 21 Days: PND -21
  - Lactation 21 Days: PND 0
  - Daily Cocaine Injections: PND 21, 54, 60

- **Events**:
  - Dams Bred
  - Pups Born
  - Weaning
  - FPA Testing

- **Details**:
  - 0, 3, or 6 mg/kg/day PCBs (n=8 dams/PCB exposure group)
  - Two male and two female offspring retained/litter (n=16 males and 16 females/PCB exposure group)
  - 20 mg/kg (IP) cocaine given to one male and one female offspring/litter (n=8 males and 8 females/PCB exposure group)
  - Second male and second female from each litter did not receive repeated daily injections of cocaine (n=8 males and 8 females/PCB exposure group)
  - DA release measured during baseline, during assessment of DAR sensitivity, in response to 20 mg/kg cocaine (IP), and during continuous stimulation in all offspring.
Figure 2. Dopamine release evoked by a test stimulus (T1) was compared with DA release to a second test stimulus (T2), the latter of which was preceded by 5-80 conditioning pulses delivered 0.3 seconds prior to T2. The data above represent attenuation measured after 80 conditioning pulses and are presented as a percent of T1 stimulus (T2/T1*100) with higher dopamine autoreceptor (DAR) sensitivity represented by a lower T2 amplitude relative to T1. DAR sensitivity increased in a PCB dose-dependent manner for the males but not the females. Females exhibited significantly greater DAR sensitivity than the males in the 0 and 6 mg/kg/day exposure groups, with a similar trend observed in the 3 mg/kg/day PCB exposure group (n=8 single injection + 8 repeated injection rats/sex/exposure group). Error bars represent the standard error of the mean; (a) p < .05, (b) p < .01.

216x152mm (300 x 300 DPI)
Following a single IP cocaine injection (20 mg/kg), rats perinatally exposed to 6 mg/kg/day PCBs had a significantly higher percent increase in peak DA release (relative to baseline) compared to the percent increase that occurred in the controls. Likewise, rats exposed to 3 mg/kg/day PCBs also exhibited a percent increase in peak DA release, but the difference from that observed in controls only approached significance. Following the last of seven daily IP injections of cocaine (also 20 mg/kg), rats exposed to the 3 mg/kg/day had a significantly lower percent increase in peak DA release compared to what was observed in the controls. Control rats given repeated cocaine injections exhibited a significantly higher percent increase in DA release compared to control rats given a single cocaine injection – an effect not found in the 3 or 6 mg/kg/day PCB groups (n=8 males + 8 females/injection schedule/PCB exposure group). Error bars represent the standard error of the mean; (a) p = .055, (b) p < .05, (c) p <.01.
Figure 4: Dopamine release in nucleus accumbens core one hour after cocaine injection and following continuous stimulation of the medial forebrain bundle. The sum of oxidation current (nAmps) was measured in all three exposure groups beginning 30 seconds and ending 90 seconds after the start of continuous stimulation (n = 8 single injection males + 8 repeated injection males + 8 single injection females + 8 repeated injection females/PCB exposure group). Error bars represent the standard error of the mean; (a) p= .047.

146x99mm (300 x 300 DPI)