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A Comparison of Presynaptic and Postsynaptic Dopaminergic Agonists on Inhibitory Control Performance in Rats Perinatally Exposed to PCBs

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Abstract

Polychlorinated Biphenyls (PCBs) are very stable environmental contaminants whose exposure induces a number of health and cognitive concerns. Currently, it is well known that PCB exposure leads to poor performance on inhibitory control tasks. It is also well known that dopamine (DA) depletion within medial prefrontal cortex (mPFC) leads to poor performance on inhibitory control tasks. However, what is not well established is whether or not the inhibitory control problems found following PCB exposure are mediated by DA depletion in mPFC. This study was an investigation into the link between perinatal exposure to PCBs, the effect of this exposure on DA neurotransmission in the mPFC, and inhibitory-control problems during adulthood using a rodent model. The current study served to determine if microinjections of different DA agonists (the presynaptic DA transporter inhibitor and vesicular monoamine transporter agonist bupropion, the postsynaptic DA receptor 2 (DAD2) agonist quinpirole, and the postsynaptic DA receptor 1 (DAD1) agonist SKF81297) directly into the mPFC would differentially improve performance on an inhibitory control task in rats perinatally exposed to an environmentally relevant PCB mixture. Findings suggest several significant sex-based differences on differential reinforcement of low rates (DRL) 15 performance as well as some evidence of differential effectiveness of the DA agonists based on PCB exposure group.

Keywords

DRL15; Bupropion; SKF81297; Quinpirole; Sex Differences; Dopamine

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1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous man-made substances that have been shown to cause numerous health problems, including cancers and other deleterious effects on the reproductive, nervous, and endocrine systems (ATSDR, 2000; Langer et al., 2009; Rosin & Martin, 1981; Sazonova et al., 2011; Schantz et al., 1997; Seegal, 1994; Selvakumar et al., 2013; Shen et al., 2011; Tilson, & Kodavanti, 1997; Vitalone et al., 2010). When human PCB exposure occurs, a body-burden of toxicity develops that can affect the developing embryo and fetus of a pregnant woman (Berk 2013; Guvenius et al., 2003). Exposure from mother to offspring occurs not only during gestation but also during lactation, as PCBs readily accumulate in breast milk (Faroon et al., 2000; Guvenius et al., 2003; Hansen, 1999; Jacobson et al., 1984).

Several empirical studies over the last twenty years have enhanced the understanding of how perinatal exposure to PCBs is hazardous to the nervous system. Reviews (Faroon et al., 2000; Fonnum & Mariussen, 2009) note that alterations in DA levels, caused by disruptions in several steps of neurotransmission, are commonly reported in rats and monkeys following PCB exposure; there are direct effects of exposure to PCBs on several steps of DA neurotransmission, including the synthesis, storage, release, binding, and reuptake of DA. The alterations in brain DA levels seem to depend on the type of PCB exposure and when in the lifespan exposure occurs. For example, *perinatal* exposure to the commercial PCB mixture Aroclor 1016 increased DA tissue levels in samples dissected from several brain regions including the substantia nigra, caudate nucleus, and nucleus accumbens (NAcc) in rats (Seegal, 1994). Perinatal exposure to individual PCB congeners in rodents had opposite effects on DA concentrations with exposure to the *ortho*-substituted (i.e., non-coplanar) congener PCB 47 causing a significant decrease in DA tissue concentration in the caudate nucleus and frontal cortex, while exposure to coplanar congener PCB 77 results in an increase in DA tissue levels in these same brain regions (Seegal et al., 1997). Following *adult* exposure, mixtures of mostly non-coplanar PCB congeners have been shown to decrease DA tissue concentrations in the striatum of rats (Seegal et al., 2002) and non-human primates (Seegal, 1996; Seegal et al., 1991). *In vitro* studies conducted using rat synaptosomes indicate an increase in DA release following exposure to Aroclor 1254 (Mariussen & Fonnum, 2001).

The specific mechanism responsible for the PCB-induced alterations in DA levels has been examined. Seegal and Shain (1992) reported that developmental exposure to a variety of PCBs congeners inhibited the function of a) the enzyme tyrosine hydroxylase which is the rate-limiting enzyme involved in DA synthesis, b) the dopamine transporter (DAT) which is a protein involved in DA reuptake, and c) the vesicular monoamine transporter (VMAT2) which is a protein involved in DA vesicular packaging in the presynaptic neuron. Thus, evidence from empirical studies has strongly suggested that exposure to PCBs affects the typical functioning of important presynaptic proteins and enzymes that are necessary for DA neurotransmission. However, disruption on the presynaptic side of DA neurotransmission may only be part of the reason why DA levels change following perinatal exposure to PCBs; the typical functioning for several key DA receptors (DADR) on the postsynaptic neuron may also be impaired. Empirical research investigating the effects of perinatal exposure to

PCBs on the functioning of postsynaptic DA receptors 1 and 2 (DAD1 and DAD2) is currently very limited. Coccini et al. (2011) conducted a saturation binding study and found that DA affinity for DAD1 and DAD2 receptors were diminished in cortex and striatum for both male and female weanling rat pups following perinatal exposure to the *ortho*-substituted PCB 153. However, another study, conducted by Roth-Härer et al. (2001), found that there are no effects on DAD1 or DAD2 concentration or affinity following exposure to the coplanar congener PCB 77.

A number of studies have demonstrated the importance of DA in the prefrontal cortex on various types of executive function, including inhibitory control. Reviews by Robbins and Arnsten (2009) as well as Cools and D'Esposito (2011) illustrate that monoamines, including DA, affect behavior on a number of executive functioning tasks. The current study focused on PCB-induced DA dysfunction in the medial prefrontal cortex (mPFC) because there is substantial evidence from animal studies (Brake et al., 2000; Dent, & Neill, 2012; Sable et al., 2009; Schoffemeer et al., 2011; Sokolowski & Salamone, 1994) that suggest a link between DA functioning in this area and inhibitory control performance. For example, Grégoire et al. (2012) found that both inhibitory control performance and expression of DAD1 receptors in regions of PFC in rats were impaired in a dose-dependent manner following administration of *d*-amphetamine – a drug known to increase synaptic DA by inhibiting DAT (Kahlig & Galli, 2003). An earlier study by Sokolowski and Salamone (1994) used 6-hydroxydopamine (6-OHDA) lesions to deplete DA in mPFC and found that performance on a differential reinforcement of low rates (DRL) of responding task was impaired. Similarly, Peterson et al. (2003) also found impaired DRL performance in rats during amphetamine withdrawal – an effect argued to occur due to decreased DA function in the mPFC. Deficits in inhibitory control have consistently been found following exposure to PCBs in humans (Stewart et al., 2003; Stewart et al., 2006), non-human primates (Rice, 1998), and rodents (Sable et al., 2009) when tested on inhibitory control tasks, such as a DRL task. These deficits in DRL performance are believed to result from depleted DA in mPFC caused by perinatal PCB exposure (Sable et al., 2009).

In this study, three different DA agonists (described below) were directly administered into the mPFC of PCB-exposed rats in an attempt to facilitate different aspects of dopaminergic transmission and promote recovery of inhibitory control function. In theory, synaptic DA in PCB-exposed animals, and therefore inhibitory control performance, could potentially be augmented by drugs that act as either a DAT inhibitor and VMAT2 agonist, a DAD1 agonist, or a DAD2 agonist. For the first group, the drug bupropion was microinjected directly into mPFC; bupropion functions as a DA agonist by both inhibiting DAT (Dwoskin et al., 2006; Learned-Coughlin et al., 2003) as well as increasing the function of VMAT2 (Rau et al., 2005).

Postsynaptic DA functioning in PCB-exposed animals could alternatively be augmented by drugs that act as postsynaptic DA receptor agonists. Thus, a DAD1 (SKF81297) and DAD2 (quinpirole) agonist were also individually microinjected directly into mPFC to determine if enhanced DAD1 and/or DAD2 receptor activation could remedy PCB-induced deficits on inhibitory control performance. *In vitro* studies using SKF81297 show a dose-dependent effect on DA concentrations in the PFC and striatum (Dumont et al., 2004). Likewise, *in*

vitro administration of quinpirole also increases synaptic DA levels in rat brain regions including the mPFC (Levant et al., 1993; Rodrigo et al., 2011).

In sum, it is well known that PCB exposure leads to poor inhibitory control performance. It is also well known that DA depletion within mPFC leads to poor inhibitory control performance. What is not well established is whether or not the inhibitory control problems found following PCB exposure are mediated by DA depletion in mPFC. The purpose of this study was to examine the effects of microinjections of bupropion, quinpirole, and SKF81297 directly into the mPFC on inhibitory control performance in adult rats that were perinatally exposed to an environmentally relevant PCBs mixture. Inhibitory control performance was measured using a DRL 15 task. A deficit in DRL performance following perinatal PCB exposure was expected. It was hypothesized that microinjections of the chosen DA agonists would improve inhibitory control performance in PCB-exposed animals by increasing endogenous synaptic DA levels and by activation of postsynaptic DA receptors in the mPFC.

2. Methods

2.1 Subjects

Cohorts of nulliparous female Long-Evans rats (Harlan) were housed in standard rat shoe-box caging. All rats were given water ad libitum and fed a diet of Harlan Teklad 2020X rat chow (a high-protein, low phytoestrogen diet) in order to maintain overall rodent health and to ensure that the pregnant females were able to support their litters. All rats were kept on a regular 12 hour light/dark cycle (lights on at 0730 hr). Rats were dosed, weighed, tested, and fed at the same times each day, during the lights-on phase of the cycle.

2.2 Exposure

The female rats were delivered to the housing facility and housed individually upon arrival. Dosing procedures, including a rationale for the doses selected have previously been explained in detail (Kostyniak et al., 2005; Sable et al. 2006; 2009; 2010). Briefly, a stock solution of the Fox River PCB mixture (Kostyniak et al., 2005) was diluted with corn oil to make three dosing solutions: 0 $\mu\text{L}/\text{mL}$ PCBs (corn oil only), 7.5 $\mu\text{L}/\text{mL}$ PCBs, and 15.0 $\mu\text{L}/\text{mL}$ PCBs. The stock solution was pipetted daily onto one-half of a vanilla wafer cookie at a volume of 0.4 mL/kg to yield final doses of 0, 3, and 6 mg/kg/day. The concentration of PCBs in the dosing solutions was verified via gas chromatography–mass spectrometry prior to dosing. The female rats were presented with their respective PCB dose (via the dosed cookie) for 28 consecutive days in order to develop a PCB body burden. On day 29, each female rat was paired with one unexposed male Long-Evans rat. Breeding pairs remained together for 8 days, and the female was removed from the breeding cage for PCB dosing for a maximum of 20 minutes each day to ensure the dosed cookie had been eaten. After breeding, the pairs were separated and females continued to be dosed during gestation (roughly 21 days) and lactation (21 days).

To ensure an even distribution of exposure, the number of pups reared by each dam after birth was controlled by eliminating litters that were too small and culling large litters; litters that had fewer than eight pups were eliminated, and large litters were culled to 10 pups, with

the extra pups cross-fostered into litters of the same age and exposure group in order to increase any litters of 8 pups to 10 pups. Cross-fostered pups were marked by ear clip and were not used for later testing. Elimination of small litters occurred at an equal rate for all exposure groups; this fact, plus consistent null findings of any effect of PCB exposure on the number of implantation sites relative to the number of pups born, reduced concerns that small litters were the direct result of higher levels of PCB exposure. Weaning occurred on postnatal day (PND) 21, at which time PCB exposure ended. The procedures above resulted in 18, 21, and 20 usable litters from the 0, 3, and 6 mg/kg/day PCB groups, respectively. At weaning, one male/female pair from each litter was sacrificed (when available) to determine physiological measures of toxicity. Additional male/female littermate pairs were kept for behavioral testing.

Physiological measures, including the dam liver weight, dam implantation sites, and brain:body weight, liver:body weight, and thymus:body weight ratios in the pups at the time of weaning, were determined to confirm PCB exposure (see Sable et al., 2010). Other general developmental outcomes, including the percent gestational and lactational weight gain, and postnatal body weights of the pups on PND 0, 7, 14, and 21 were also collected.

2.3 Procedure

When the offspring were 90 days old, they were placed on an IACUC-approved food restriction schedule of 85% of their free-feeding weight which persisted until the end of the experiment. At 100 days old, rats began training and testing in the operant chambers. Operant testing occurred six days/week, and food restriction procedures occurred seven days/week.

2.3.1 Apparatus—Behavioral testing was conducted in 10 automated operant testing chambers that were both sound-reducing and ventilated and were outfitted with two retractable levers, cue lights, house light, and food magazine. All operant programs were controlled via a PC equipped with Med-PC IV software (Med Associates).

2.3.2 Autoshaping and fixed ratio training—The details of these procedures are provided elsewhere (Sable et al., 2006; 2009). During both phases every press on either lever resulted in the delivery of a 45 mg reward pellet (Bio Serv; Frenchtown, New Jersey). Rats remained on autoshaping until 100 lever presses occurred in a session and zero free pellets were dispensed. Fixed-ratio training lasted for three sessions.

2.3.3 Differential reinforcement of low rates (DRL)—The details of the DRL procedure are also explained elsewhere (Sable et al., 2006; 2009). Each DRL session continued until 90 min had elapsed or the rat earned 200 reinforcers. After two sessions of DRL 1 (i.e., 1 sec required between lever presses), the rats progressed through two sessions of DRL 5 and two sessions of DRL 10. Finally, rats were tested on a DRL 15 program over 35 days to promote steady-state performance. The DRL progression used was selected to be consistent with previous studies showing PCB-associated deficits (Sable et al., 2006; 2009).

2.3.4 Surgery—After steady-state performance on DRL 15 was established, each rat underwent bilateral implantation surgery with stainless-steel guide cannulae aimed at the

mPFC. The guide cannulae were 26 gauge (Plastics One; Roanoke, Virginia) through which either dummy cannula or implanting cannula (both 33 gauge, also from Plastics One) were inserted. The implanting cannulae were placed inside the cannula guides during surgery to prevent occlusion of the guide cannulae during implantation. The dummy cannula served a similar purpose after the guides were implanted, and were only removed during microinjection.

Surgeries was performed using a bilateral stereotaxic with a mix of oxygen and Isothesia (isoflurane 1%) delivered to the stereotax for anesthesia. Three skull screws (Plastics One; Roanoke, Virginia) were placed in the skull posterior to bregma to anchor the dental sealant that was applied later. The cannula guides were implanted at a 10° angle at +3 mm A/P and +1.4 mm M/L to bregma and lowered to -2.7 mm ventral from the skull surface. Herculite dental sealant was applied and cured around the skull screws and cannulae to create a cap. Rats were given subcutaneous injections of Baytril (an antibiotic, 2.5 mg/kg) and carprofen (an anti-inflammatory, 2.5 mg/kg), and were allowed 24 hrs for recovery with an additional intraperitoneal injection of carprofen 48 hrs post-surgery.

2.3.5 Drugs—Bupropion hydrochloride was mixed in 1:10 double distilled H₂O solution:physiological saline at aliquot doses of 0.02 mg/mL (72 µM), 0.12 mg/mL (434 µM), and 0.24 mg/mL (869 µM). Bupropion ($K_i = 409\text{--}831$ nM) has been reported to have a DAT inhibitor potency that approximates cocaine ($K_i = 270\text{--}690$ nM) but without the positive highs/euphoria associated with cocaine (Heal et al., 2014). Thus, the doses of bupropion selected were similar to doses of cocaine (i.e., approximately 165 – 659 µM), which, when microinjected into mPFC, successfully reinstated operant cocaine seeking behavior (Park et al., 2001). The microinjections of bupropion doses in the current study (0.0 µg/side, 0.01 µg/side, 0.06 µg/side, 0.12 µg/side) were given in ascending order (one dose/session/day) with three replicates of each dose.

SKF81297 hydrobromide was mixed in 1:10 dimethyl sulfoxide (DMSO) solution:physiological saline at aliquot doses of 0.12 mg/mL (0.3 mM), 0.24 (0.6 mM), and 0.40 (1.0 mM). The doses of SKF81297 selected were similar to those used by Gurden, Takita, and Jay (2000) who found that local infusions of .1 and 1 mM SKF81297 *in vivo* into mPFC increased D1 receptor populations and long term potentiation within PFC. Microinjections of SKF81297 doses in the current study (0.0 µg/side, 0.06 µg/side, 0.12 µg/side, 0.20 µg/side) were given in ascending order (one dose/session/day) with three replicates of each dose.

Quinpirole hydrochloride was mixed in 1:10 double distilled H₂O solution:physiological saline at aliquot doses of 0.2 mg/mL (782 µM), 1.0 mg/mL (3910 µM), and 2.0 mg/mL (7819 µM). The highest dose was similar to one used by Yates et al. (2014), who found that a 9774 µM dose of quinpirole microinjected directly into the mPFC altered impulsive choice as measured via a delay discounting task. Microinjections of quinpirole doses in the current study (0.0 µg/side, 0.01 µg/side, 0.05 µg/side, 1.0 µg/side) were given in ascending order (one dose/session/day) with three replicates of each dose.

2.3.6 Dopamine agonist drug challenges—All rats were given 24 hr to recover from surgery before beginning microinjections. The rats were first given a 3-day series of mock injections to become acclimated to the microinjection procedure. During the mock injections, the dummy cannulae were removed and the microinjection cannulae (33 gauge) was inserted into each guide to protrude 1 mm below the bottom of the guides. The pump was driven for 30 s but no vehicle or drug solution was administered. The microinjectors remained in place inside each cannula guide for one minute (30 s with pump running, 30 s without pump running) before they were removed and the dummy cannulae were replaced. Rats were not tested on DRL 15 following the first day of mock injections, but were tested following the second and third mock injections. On the following days, microinjections of the specified dopamine agonist for each rat were administered immediately prior to DRL 15 testing. Each male-female pair only received microinjections of either bupropion, or SKF81297, or quinpirole, and duplicate male-female pairs (i.e., pairs from the same litter) always received a different DA agonist. Microinjections were given for 30 s at a rate of 1 μ L/min. Microinjectors remained in place for another 30 s to allow for diffusion before being removed, after which the dummy cannula were replaced and the rat was immediately placed in the operant chamber for DRL 15 testing.

2.3.7 Implantation site verification—After completing all three replicates of each dose for the specified dopamine agonist, rats were euthanized via overexposure to carbon dioxide. Methylene blue dye was posthumously microinjected at a rate of 1 μ L/min into the mPFC in order to verify the locations of drug microinfusion. Brain tissue was sliced in a cryostat at a thickness of 20 microns to determine if the drug microinjections occurred in the prelimbic mPFC. If the dye revealed that the bilateral microinjection sites were not in mPFC then data from the rat was not included in the analyses. According to an analysis of the breath of brain tissue affected by a variety of volumes of bromophenol blue (a dye that is commonly used in a similar manner to methylene blue), Myers (1966; as cited in Meyers, 1971) found that injections at a rate of 1 μ L/min dyed 1.1 mm of tissue surrounding the microinjector. Based on this information and visual confirmation of the methylene blue dye it was assumed that only tissue within the mPFC was affected by the injections of the DA agonists. Only data from rats whose brain tissue displayed the proper placement for cannulae implantation were included in our analysis.

3. Data Analysis

A list of all of the dependent measures that were analyzed for this study is included in Table 1. All data analyses were conducted via SPSS for Windows, version 20.

3.1 Physiological measures

Percent gestational and lactational weight gain, dam liver:body weight ratio, and number of implantation sites were analyzed via one-way between-subjects ANOVAs for PCB exposure group. The pup organ:body weight ratio data was analyzed separately for brain, liver, and thymus via 3×2 (PCB group $\times$ sex) mixed ANOVA. The data on pup postnatal weight gain was similarly analyzed but also included day (PND 0, 7, 14, and 21) as an additional repeated-measures factor. The averages for these physiological measures were calculated for

all dams/pups within the same litter to determine the effect of exposure to PCBs on overall health.

3.2 Baseline DRL 15 performance

3.2.1 General learning outcomes—The ratio of reinforced to nonreinforced lever presses and the number of reinforcers earned were used as overall measures of performance; a higher ratio of reinforced:nonreinforced lever presses and a higher number of reinforcers earned was considered to be indicative of better performance. Data across the 35 days of testing was averaged into seven 5-day blocks. These data were analyzed separately via $3 \times 2 \times 7$ (PCB exposure group $\times$ sex $\times$ 5-day block) mixed ANOVAs. This measure of baseline performance was calculated for all male/female littermate pairs tested. However, the data from same-sex littermates was averaged to yield only one male value and one female value per litter (to eliminate potential litter effects).

3.2.2 Response pattern analysis—Each lever press that was made during testing in an attempt to earn a reinforcer (i.e., those that are not made to initiate a trial), was categorized into one of eight inter-response time (IRT) bins each 2.49 s in size (e.g., 0–2.49 s, 2.50–4.99 s, 5.00–7.49 s, 7.50–8.99 s, 9.00–12.49 s, 12.50–14.99 s, 15.00–17.49 s, and > 17.50 s) and was then analyzed via $3 \times 2 \times 7 \times 8$ (PCB exposure group $\times$ sex $\times$ 5-day block $\times$ IRT bin) mixed ANOVAs. As before, data from multiple same-sex littermates was averaged to eliminate potential litter effects.

3.3 Dopamine agonist drug challenge

3.3.1 Change in general learning outcomes—The effect of each DA agonist on DRL 15 performance (i.e., ratio of reinforced:nonreinforced lever presses and reinforcers earned), was determined by averaging each of these measures of DRL performance across the three replicates of each drug dose. Each measure was analyzed with $3 \times 2 \times 4$ (PCB exposure group $\times$ sex $\times$ DA agonist dose) mixed ANOVAs.

3.3.2 Change in response pattern analysis DRL 15—The effect of each DA agonist on the proportion of responses within each of the eight IRT bins was determined by first averaging across the three replicates of each drug dose. The change in the proportion of responses within each of the eight IRT bins was analyzed using a $3 \times 2 \times 4 \times 8$ (PCB exposure group $\times$ sex $\times$ DA agonist dose $\times$ IRT) mixed ANOVA.

Additional post hoc Dunnett *t*-tests and simple contrasts were conducted as appropriate to determine the nature of significant main effects and interactions found in the previously described omnibus analyses.

4. Results

4.1 Physiological Measures

4.1.1 Dam exposure physiological effects—There was no effect of PCB exposure on dam liver:body weight ratio, $F(2, 56) = 0.650$, $p = .526$, number of implantation sites, $F(2,$

56) = 2.153, $p = .718$, percent gestational weight gain, $F(2, 56) = .596$, $p = .555$, or percent lactational weight gain, $F(2, 56) = .044$, $p = .957$ (see Table 2).

4.1.2 Pup postnatal weight gain—As expected, there was a significant main effect of sex on pup postnatal weight gain, $F(1, 56) = 25.443$, $p < .001$, where the average weight of the males was significantly higher than that of females. Additionally, a significant day x PCB exposure group interaction, $F(6, 168) = 12.053$, $p < .001$, revealed that male and female pups from the 3 mg/kg/day and 6 mg/kg/day PCB dose groups were significantly lighter than the control pups on PNDs 7, 14, and 21. See Table 3 for means and *SEMs* for pup postnatal weight gain.

4.1.3 Pup developmental mortality analyses—There were no significant effects of perinatal PCB exposure on litter size, $F(2,58) = .452$, $p = .639$, the percent of males born per litter, $F(2,58) = .098$, $p = .907$, the percent of live pups born on PND0, $F(2,58) = 1.318$, $p = .276$, or the percent of pups that died between PND0 and PND21, $F(2,58) = 1.078$, $p = .347$ (see table 4).

4.1.4 Pup organ weight analyses—There was a significant main effect of sex on brain weight at PND 21, $F(1, 56) = 9.419$, $p = .003$, wherein females had significantly lighter brains than males. There was also a significant main effect of PCB exposure group on brain weight, $F(2, 56) = 5.986$, $p = .004$, wherein pups from the 6 mg/kg/day PCB group had significantly lighter brains than the control pups ($p = .004$). There was a significant main effect of sex on liver weight at PND 21, $F(1, 56) = 10.979$, $p = .002$, wherein females had significantly lighter livers than males. There was also a significant main effect of PCB exposure group on liver weight, $F(2, 56) = 21.498$, $p < .001$, wherein PCB exposed pups had significantly heavier livers than the control pups (both at $p < .001$). There was a significant main effect of PCB exposure group on thymus weight at PND 21, $F(2, 56) = 29.680$, $p < .001$, wherein PCB exposed pups had significantly lighter thymi than the control pups (both at $p < .001$). Means and *SEMs* of the pup organ weights are displayed in Table 5.

4.1.5 Pup organ:body weight ratios—There was a significant main effect of PCB exposure group on brain:body weight ratio, $F(2, 56) = 9.588$, $p < .001$, on liver:body weight ratio, $F(2, 56) = 106.806$, $p < .001$, and on thymus:body weight ratio, $F(2, 56) = 21.007$, $p < .001$. Exposed pups from the 3 mg/kg/day and 6 mg/kg/day PCB dose groups had significantly higher brain:body weight ($p = .005$) and liver:body weight ($p < .001$) ratios than the control pups at the time of weaning. Pups exposed to 3 mg/kg/day and 6 mg/kg/day also had significantly reduced thymus:body weight ratios than the control pups at weaning ($p = .001$ and $p < .001$, respectively) at weaning. Means and *SEMs* for the organ:body weight ratios are displayed in Table 6.

4.2 Steady-state DRL 15 Measures

4.2.1 General learning outcomes—There was a significant main effect of sex on the ratio of reinforced:nonreinforced lever presses, $F(1, 56) = 6.149$, $p = .016$, wherein females ($M = .682$, $SEM = .040$) had a significantly higher ratio than males ($M = .555$, $SEM = .037$). Further, there was a significant sex x exposure group interaction, $F(2, 56) = 4.456$, $p = .015$,

wherein control females had a significantly higher ratio of reinforced:nonreinforced lever presses than control males ($p = .036$) and control females had a significantly higher ratio compared to the 3 mg/kg/day exposed females ($p = .014$). The 6 mg/kg females appeared to have a significantly higher ratio of reinforced:nonreinforced responses than males in the same exposure group, but this difference was not statistically significant ($p = .651$) (see Figure 1a). There was a significant sex x exposure group interaction on number of reinforcers earned, $F(2, 56) = 5.083$, $p = .009$, wherein control females earned significantly more reinforcers than control males ($p = .002$) and the 3 mg/kg/day females ($p = .048$) (see Figure 1b). Males and females did not differ in the 3 mg/kg/day or 6 mg/kg/day PCB groups. A significant sex x block interaction, $F(6, 336) = 3.415$, $p = .003$, revealed that females had a significantly higher ratio of reinforced:nonreinforced lever presses than males in the fifth ($p = .013$), sixth ($p = .024$), and seventh ($p = .018$) five-day testing blocks (see Figure 2).

4.2.2 Response pattern analysis—A significant IRT bin x exposure group interaction was found, $F(14, 392) = 1.841$, $p = .031$, wherein the 3 mg/kg/day PCB-exposed rats exhibited a significantly higher proportion of responses in the 0.0–2.49 s time bin ($p = .013$) and a significantly lower proportion of responses in the 15.0–17.49 s ($p = .031$) time bin compared to control rats (see Figure 3). A significant sex x IRT bin x exposure group interaction was found, $F(14, 392) = 3.209$, $p < .001$. Control males had a significantly higher proportion of responses in the 5.0–7.49 s ($p = .049$), 7.5–8.99 s ($p = .002$), 9.0–12.49 s ($p < .001$), 12.5–14.99 s ($p = .001$) time bins and a significantly lower proportion of responses in the 15.0–17.49 s ($p = .019$), and 17.5 s or greater ($p = .003$) time bins compared to control females. Control males also had a significantly lower proportion of responses in the 0.0–2.49 s ($p = .018$) time bin and a significantly higher proportion of responses in the 12.5–14.99 s ($p = .047$) time bin compared to the 3 mg/kg/day PCB-exposed males. The control females also exhibited a significantly higher proportions of responses in the 17.5 s or greater ($p = .008$) time bin compared to the 3 mg/kg/day PCB-exposed females. Males from the 6 mg/kg/day exposure group had a significantly higher proportion of responses in the 12.5–14.99 s ($p = .047$) time bin and a significantly lower proportion of responses in the 17.5 s or greater ($p = .002$) time bin compared to 6 mg/kg/day females (see Figure 4). Finally, a significant sex x IRT bin interaction was also found, $F(7, 392) = 3.209$, $p < .001$, wherein males showed a significantly higher proportion of responses in the 9.0–12.49 s ($p = .014$) time bin and a significantly lower proportion of responses in the 15.0–17.49 s ($p = .011$), and the 17.5s or greater ($p = .016$) time bins compared to females (sup. Figure 1).

4.3 Bupropion Drug Challenge

4.3.1 Change in general learning outcomes—There was a significant main effect of bupropion dose on the ratio of reinforced:nonreinforced lever presses, $F(3, 63) = 9.872$, $p < .001$, with .06 µg/side ($M = .840$, $SEM = .062$; $p = .002$) and .12 µg/side ($M = .901$, $SEM = .070$; $p = .002$) resulting in a higher ratio relative to 0 µg/side ($M = .725$, $SEM = .049$).

There was a significant dose x exposure group interaction on the number of reinforcers earned, $F(6, 63) = 2.317$, $p = .044$, wherein bupropion led to a significant increase in the number of reinforcers earned for the control animals at the .01 µg/side ($p = .047$) and .12 µg/

side ($p = .019$) doses compared to the 0 $\mu\text{g}/\text{side}$ dose. Likewise, rats exposed to 6 $\text{mg}/\text{kg}/\text{day}$, but not the 3 $\text{mg}/\text{kg}/\text{day}$, also showed improvement after receiving the .06 and .12 $\mu\text{g}/\text{side}$ dose ($p = .003$ and $p = .002$, respectively; see Figure 5).

4.3.2 Change in response pattern analysis—There was a significant dose $\times$ IRT bin interaction for bupropion, $F(21, 441) = 6.845$, $p < .001$. The .01 $\mu\text{g}/\text{side}$ dose led to a decrease in the proportion of responses in the 5.0–7.49 s ($p = .014$) and 7.5–8.99 s ($p = .001$) IRT bins relative to 0 $\mu\text{g}/\text{side}$. The .06 $\mu\text{g}/\text{side}$ dose led to a decrease in the proportion of responses in the 5.0–7.49 s ($p = .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p = .004$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p = .004$) IRT bin relative to 0 $\mu\text{g}/\text{side}$. The .12 $\mu\text{g}/\text{side}$ dose led to a decrease in the proportion of responses in the 2.5–4.99 s ($p = .013$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p < .001$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p = .002$) IRT bins relative to 0 $\mu\text{g}/\text{side}$.

Additionally, there was a significant sex $\times$ dose $\times$ IRT bin $\times$ exposure group interaction, $F(42, 441) = 1.720$, $p = .004$, wherein control females showed a significant increase in the proportion of responses in the 15.0–17.49 s time bin when compared to the 3 $\text{mg}/\text{kg}/\text{day}$ PCB females that were microinjected with the .01 $\mu\text{g}/\text{side}$ bupropion dose ($p = .008$), the .06 $\mu\text{g}/\text{side}$ bupropion dose ($p = .002$), and the .12 $\mu\text{g}/\text{side}$ bupropion dose ($p < .001$). Control females also showed a significant increase in the proportion of responses in the 15.0–17.49 s time bin when compared to the 6 $\text{mg}/\text{kg}/\text{day}$ PCB females that were microinjected with the .06 $\mu\text{g}/\text{side}$ bupropion dose ($p = .025$) and the .12 $\mu\text{g}/\text{side}$ bupropion dose ($p = .001$). The males did not show this same trend, and no significant effects were found (sup. Figure 2).

4.4 SKF81297 Drug Challenge

4.4.1 Change in general learning outcomes—As expected, there was a significant main effect of SKF81297 dose on the ratio of reinforced:nonreinforced lever presses, $F(3, 60) = 16.152$, $p < .001$ with an increase following .06 $\mu\text{g}/\text{side}$ ($p = .002$; $M = .507$, $SEM = .043$), .12 $\mu\text{g}/\text{side}$ ($p < .001$; $M = .619$, $SEM = .046$), and .20 $\mu\text{g}/\text{side}$ ($p < .001$; $M = .705$, $SEM = .060$) relative to the 0 $\mu\text{g}/\text{side}$ dose ($M = .395$, $SEM = .040$). There was also a significant main effect of sex on the ratio of reinforced:nonreinforced lever presses following microinjections of SKF81297, $F(1, 20) = 6.310$, $p = .021$, wherein females ($M = .646$, $SEM = .056$) had a significantly higher ratio than males ($M = .474$, $SEM = .048$).

There was a significant main effect of SKF81297 dose on the number of reinforcers earned, $F(3, 60) = 30.564$, $p < .001$, with an increase following .06 $\mu\text{g}/\text{side}$ ($p < .001$; $M = 105.754$, $SEM = 4.635$), .12 $\mu\text{g}/\text{side}$ ($p < .001$; $M = 113.297$, $SEM = 4.026$), and .20 $\mu\text{g}/\text{side}$ ($p < .001$; $M = 115.040$, $SEM = 4.463$) relative to the 0 $\mu\text{g}/\text{side}$ dose ($M = 95.080$, $SEM = 5.129$). Finally, there was also a significant main effect of sex, $F(1, 20) = 6.310$, $p = .021$, wherein females ($M = 117.232$, $SEM = 5.533$) earned significantly more reinforcers than males ($M = 98.345$, $SEM = 4.394$). No PCB-related effects were found (see Figure 6).

4.4.2 Change in response pattern analysis—There was a significant dose $\times$ IRT bin interaction for SKF81297, $F(21, 420) = 11.866$, $p < .001$. The .06 $\mu\text{g}/\text{side}$ dose led to a

decrease in the proportion of responses in 2.5–4.99 s ($p = .046$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p = .010$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p = .001$) and 17.5 s or greater ($p = .010$) IRT bins relative to 0 $\mu\text{g}/\text{side}$. The .12 $\mu\text{g}/\text{side}$ dose led to a decrease in the proportion of responses in 2.5–4.99 s ($p < .001$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p < .001$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p < .001$) and 17.5 s or greater ($p = .001$) IRT bins relative to 0 $\mu\text{g}/\text{side}$. The .20 $\mu\text{g}/\text{side}$ dose led to a decrease in the proportion of responses in 2.5–4.99 s ($p = .003$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p < .001$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p < .001$) and 17.5 s or greater ($p < .001$) IRT bins relative to 0 $\mu\text{g}/\text{side}$ (sup. Figure 3a).

There was also a significant sex $\times$ IRT bin interaction, $F(7, 140) = 2.598$, $p = .001$, wherein SKF81297 females had a significantly lower proportion of responses in the 5.0–7.49 s ($p = .007$), 7.5–8.99 s ($p = .001$), 9.0–12.49 s ($p = .001$), and 12.5–14.99 s ($p = .006$) time bins as well as a significantly higher proportion of responses in the 17.5 s or greater ($p = .008$) time bin when compared to SKF81297 males (sup. Figure 3b). No PCB-related effects were found.

4.5. Quinpirole Drug Challenge

4.5.1 Change in general learning outcomes—As expected, there was a main effect of quinpirole dose on the ratio of reinforced:nonreinforced lever presses, $F(3, 60) = 24.506$, $p < .001$, with an increase following .10 $\mu\text{g}/\text{side}$ ($p < .001$; $M = .531$, $SEM = .041$), .50 $\mu\text{g}/\text{side}$ ($p < .001$; $M = .600$, $SEM = .046$), and 1.0 $\mu\text{g}/\text{side}$ ($p < .001$; $M = .733$, $SEM = .057$) compared to the 0 $\mu\text{g}/\text{side}$ dose ($M = .389$, $SEM = .032$).

There was, as expected, a main effect of quinpirole dose on the number of reinforcers earned, $F(3, 60) = 24.506$, $p < .001$, with the .10 $\mu\text{g}/\text{side}$ dose ($p < .001$; $M = 109.792$, $SEM = 3.982$), .50 $\mu\text{g}/\text{side}$ ($p < .001$; $M = 116.843$, $SEM = 4.348$), and the 1.0 $\mu\text{g}/\text{side}$ dose ($p < .001$; $M = 124.270$, $SEM = 3.917$) yielding significant increase in reinforcers earned compared to the 0 $\mu\text{g}/\text{side}$ dose ($M = 100.215$, $SEM = 3.862$). Moreover, there was a significant sex $\times$ dose $\times$ exposure group interaction on the number of reinforcers earned, $F(6, 60) = 2.971$, $p = .013$. The increase in quinpirole dose from 0 $\mu\text{g}/\text{side}$ to .0.10 $\mu\text{g}/\text{side}$ ($p = .016$) and from 0 $\mu\text{g}/\text{side}$ to 1.0 $\mu\text{g}/\text{side}$ ($p = .031$) led to a significant increase in the number of reinforcers earned for control males. The increase in quinpirole dose from 0 $\mu\text{g}/\text{side}$ to .0.1 $\mu\text{g}/\text{side}$ ($p = .005$), from 0 $\mu\text{g}/\text{side}$ to 0.5 $\mu\text{g}/\text{side}$ ($p = .009$), and from 0 $\mu\text{g}/\text{side}$ to 1.0 $\mu\text{g}/\text{side}$ ($p < .001$) led to a significant increase in the number of reinforcers earned for 3 mg/kg/day males. The increase in quinpirole dose from 0 $\mu\text{g}/\text{side}$ to .0.10 $\mu\text{g}/\text{side}$ ($p = .046$), from 0 $\mu\text{g}/\text{side}$ to 0.50 $\mu\text{g}/\text{side}$ ($p = .041$), and from 0 $\mu\text{g}/\text{side}$ to 1.0 $\mu\text{g}/\text{side}$ ($p = .013$) led to a significant increase in the number of reinforcers earned for 6 mg/kg/day males. The increase in quinpirole dose from 0 $\mu\text{g}/\text{side}$ to 0.50 $\mu\text{g}/\text{side}$ and from 0 $\mu\text{g}/\text{side}$ to 1.0 $\mu\text{g}/\text{side}$ led to a significant increase in the number of reinforcers earned for control females ($p = .034$ and $p = .008$, respectively) and 3 mg/kg/day females ($p = .009$ and $p = .028$, respectively) (see Figure 6). The increase in quinpirole dose did not lead to an increase in the number of reinforcers earned for the 6 mg/kg/day females.

4.5.2 Change in response pattern analysis—There was a significant quinpirole dose x IRT bin interaction, $F(21, 420) = 15.463, p < .001$. The 0.10 µg/side dose led to a decrease in the proportion of responses in 2.5–4.99 s ($p = .017$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p < .001$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p < .001$) and 17.5 s or greater ($p < .001$) IRT bins relative to 0 µg/side. The 0.50 µg/side dose led to a decrease in the proportion of responses in 2.5–4.99 s ($p < .001$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p < .001$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p < .001$) and 17.5 s or greater ($p < .001$) IRT bins relative to 0 µg/side. The 1.0 µg/side dose led to a decrease in the proportion of responses in 2.5–4.99 s ($p = .001$), 5.0–7.49 s ($p < .001$), 7.5–8.99 s ($p < .001$), and 9.0–12.49 s ($p < .001$) IRT bins as well as an increase in the proportion of responses in the 15.0–17.49 ($p < .001$) and 17.5 s or greater ($p < .001$) IRT bins relative to 0 µg/side (sup. Figure 4). There were no effects of sex or PCB exposure group on the IRT analysis for quinpirole.

5. Discussion

5.1 Physiological Measures

5.1.1 Dam exposure physiological effects—Based on previously published reports for the Fox River PCB mixture (Kostyniak et al., 2005; Sable & Schantz, 2006; Sable et al., 2009), few PCB-related effects in the dams were expected. Gestational and lactational weight gain and dam liver:body weight ratio of PCB-exposed dams were not different from control dams. Likewise, no significant differences were found among the three exposure groups on the number of implantation sites relative to number of pups born, thus indicating that there was no toxic effect of PCB exposure on pup health while in utero.

5.1.2 Pup postnatal weight gain—Postnatal weight gain was expected to be lowest in PCB-exposed pups at the earlier, but not the later time points. This expectation was not fully supported; it was found in the current study that the control pups were heavier than the PCB-exposed pups on days 7, 14, and 21 of postnatal weighing. This finding indicates that pups had the same average weight at birth, regardless of PCB exposure group, but that in the days after birth the control pups gained significantly more weight than the exposed pups and maintained this weight gain throughout pre-adolescence.

5.1.3 Pup developmental mortality analyses—We found no evidence to suggest that perinatal PCB exposure led to any decrease in the number of pups born per litter, the number of still births, nor in the number of pups that died between birth and the time of weaning.

5.1.4 Pup organ weight analysis—A comparison of the organ weights at weaning between the exposure groups was used as an additional validation of indirect exposure to PCBs. The 3 mg/kg/day and 6 mg/kg/day PCB exposed pups showed evidence of PCB toxicity, including lighter brain weights, heavier liver weights, and lighter thymus weights.

5.1.5 Pup organ:body weight ratios—Organ:body weight ratios in offspring were determined in order to further illustrate the physiological effects of PCB exposure. The 3 mg/kg/day and 6 mg/kg/day PCB-exposed pups showed evidence of PCB toxicity, including

higher brain:body weight and liver:body weight ratios as well as lower thymus:body weight ratios. These findings are in line with our expectations and replicate previous research using the same dosing regimen with the Fox River PCB mixture (Sable et al., 2009; 2010)

5.2 Baseline Measures

5.2.1 General learning outcomes—Control females performed better on the DRL 15 task when compared to control males and 3.0 mg/kg PCB-exposed females. Interestingly, 6 mg/kg females exhibited better performance than their male counterparts, although the difference did not reach statistical significance. PCB-related impairments on the performance on DRL inhibitory control tasks have been previously reported (Rice, 1998; Sable & Schantz, 2006; Sable et al., 2006; Sable et al., 2009; Stewart et al., 2003; Stewart et al., 2006).

5.2.2 Response pattern analysis—PCB exposed rats exhibited a higher proportion of responses in the 0.0–2.49 s time bin and a significantly lower proportion of responses in the 15.0–17.49 s time bin compared to control rats. This finding serves as evidence for PCB-exposed rats to exhibit more inhibitory control problems than control rats. The tendency to immediately (and inappropriately) press the lever in order to try to earn a reinforcer was indicated by a higher proportion of responses in the 0.0–2.49 s time bin. Impaired performance on the task, and an inefficient means of earning food rewards, was indicated by a lower proportion of responses in the 15.0–17.49 s time bin, which is the target time for a response to be made in order to earn a reinforcer.

Control females exhibited better inhibitory control performance compared to control males, who in turn had better inhibitory control performance than PCB-exposed males. Additionally, control females also exhibited better inhibitory control performance compared to the PCB-exposed females. Females showed significantly better inhibitory control performance compared to males, even at the highest level of PCB exposure; females from the 6 mg/kg/day exposure group had a lower proportion of responses in IRT bins that were less than 15 s and a higher proportion of responses in the 17.5 s or greater IRT bin compared to 6 mg/kg/day males.

5.3 Effectiveness of Bupropion Drug Challenge

5.3.1 Change in general learning outcomes—The doses of bupropion used in this drug challenge study were found to improve performance on the DRL 15 task by increasing the ratio of reinforced:nonreinforced lever presses as well as the number of reinforcers earned. Of considerable note is that all groups of PCB-exposed rats showed a higher number of reinforcers earned following microinjections of bupropion except for 3 mg/kg/day rats. This is an interesting finding, as it suggests that bupropion was differentially effective at improving DRL performance for the different levels of PCB exposure.

5.3.2 Change in response pattern analysis—Increasing doses of bupropion led to a significant decrease in the proportion of responses in the IRT time bins spanning from 2.4–12.49 s as well as a significant increase in proportion of responses in the 15.0–17.49 time bin. The decrease in the proportion of responses found for unreinforceable time bins

following increasing doses of bupropion indicates an improved ability to inhibit premature responses. This, coupled with the increase in the proportion of responses in the reinforceable time bins, indicates that higher doses of bupropion resulted in better, more efficient performance on the DRL 15 task. This effect was most consistently found for female IRT response patterns but no significant effects were found for male IRT response patterns, indicating a potential differential effect of bupropion based on sex. These findings did not differ according to PCB exposure group.

5.4 Effectiveness of SKF81297 Drug Challenge

5.4.1 Change in general learning outcomes—Increasing doses of SKF81297 led to improved DRL 15 performance; females that received SKF81297 had a higher ratio of reinforced:nonreinforced lever presses and a higher number of reinforcers earned compared to SKF81297 treated males. These findings did not differ according to PCB exposure group.

5.4.2 Change in response pattern analysis—Increasing doses of SKF81297 led to improved inhibitory control performance and task efficiency; females microinjected with SKF81297 had a lower proportion of responses in bins ranging from 5.0–14.99 s and a higher proportion of responses in the 17.5 s or greater time bin compared to SKF81297 treated males. These findings of inhibitory control deficits did not differ according to PCB exposure group.

5.5 Effectiveness of Quinpirole Drug Challenge

5.5.1 Change in general learning outcomes—Increasing doses of quinpirole led to an increase in the ratio of reinforced:nonreinforced lever presses. No other effects were found for this measure, as there were no sex effects or PCB exposure group effects on the ratio of reinforced:nonreinforced lever presses. Numerous significant effects on the number of reinforcers earned were found following microinjections of quinpirole. As expected, there was an increase in the number of reinforcers earned for each increase in quinpirole dose. An interesting sex-specific finding following administration of the selected doses of quinpirole was the significant benefit on the number of reinforcers earned by males compared to all other DA agonists. An even more interesting effect of quinpirole administration was that increasing doses of quinpirole led to an increase in the number of reinforcers earned for all groups of males and only for the control and 6 mg/kg/day females, mirroring the potential PCB exposure-specific effect of bupropion on the number of reinforcers earned.

5.5.2 Change in response pattern analysis—Overall, it was found that increasing doses of quinpirole led to a decrease in the proportion of responses in the IRT time bins spanning from 2.5–4.99 s through 9.0–12.49 s, as well as an increase in bins greater than 15 s, thus indicating an improvement in inhibitory control. However, the doses of quinpirole selected for this study did not show a differential benefit for either sex or for any of the PCB exposure groups.

5.6. Conclusions and Suggestions for Future Research

Rats perinatally exposed to PCBs had impaired DRL performance. This study was conducted to determine whether or not microinjections into mPFC of the DA agonists

bupropion, SKF81297, and quinpirole would differentially improve inhibitory control performance in rats following perinatal exposure to PCBs. The results of this study indicated that bupropion was most effective at improving overall performance on the DRL 15 task as well as inhibitory control performance compared to the other DA agonists studied. While many of the findings reported in the current study showed significant sex-specific results, there was not much evidence to suggest that the DA agonists used in this study differentially improved performance on the DRL 15 task specifically for PCB-exposed rats when microinjected into the mPFC. Physiological outcomes clearly demonstrate the effectiveness of perinatal PCB exposure, and all measures prior to surgery indicate impaired performance on the DRL 15 task due to PCB exposure. Thus, impairments caused by PCB exposure were evident; however, the benefit of the DA agonists microinjected into mPFC used in this study for PCB-exposed rats was not evident.

Importantly, increasing doses of all three DA agonists provided a performance benefit overall with no unique pharmacological benefit to PCB-exposed animals. Rather, if anything, PCB-exposed rats were less sensitive to the doses of the chosen DA agonists. The lack of a differential effect on the PCB-exposed groups suggests that the mPFC, and the associated DA dysfunction in this area, may not be the region or mechanism solely responsible for PCB-induced reductions in DRL performance. For example, PCB-induced DA dysfunction may extend to other regions intricately involved in inhibitory control performance, particularly the striatum, the NAcc, and the hippocampus. Indeed, changes in inhibitory control performance linked to alternations in DA function within the striatum (Buckholtz et al., 2010; Neill, 1976; Pattij et al., 2014) the NAcc (Cardinal et al., 2001; Sabol et al., 2000), and the hippocampus (Abela et al., 2013) have been reported. Alterations within other monoamine systems including serotonin (Fletcher et al., 2009; Landrø et al., 2015) and norepinephrine (Bari & Robbins, 2013; Logemann et al., 2013) have also been shown to affect inhibitory control performance. PFC neurons projecting to monoamine cell bodies in the brain stem (Robbins & Arnsten, 2009) and the NAcc (Leggio et al., 2009) appear to play a neuromodulatory role by recruiting other necessary monoamine systems when necessary. This type of compensatory recruitment could potentially explain differences among treatment groups on the DRL task (i.e., why the 3 mg/kg PCB group shows impairment but the 6 mg/kg PCB group does not) as well as explain why PCB-exposed groups appeared to be less sensitive to local administration of the DA agonists into the mPFC than the control rats not exposed to PCBs. Finally, future research based on either systemic administration of the chosen DA agonists, in combination with microinjections targeting the striatum, NAcc, or hippocampus, may provide further insight.

To further clarify the findings of the current study, future studies should also aim to determine what neurochemical and neurophysiological factors might underlie differences in male and female performance on the DRL task. Specifically, it would be of interest to determine if the sex-related differences in DRL performance can be pinpointed to intrinsic factors (such as neurotransmitter levels and/or developmental organization of the brain) or if they reflect a different distribution of the cognitive resources (e.g., attention, time-keeping, response inhibition) required to successfully complete the task. It has consistently been noted that males tend to exhibit a stronger behavioral profile of impulsive action, while females tend to exhibit deficits in attentional processes and impulsive choice (Newcorn et

al., 2001). Specifically, Van Hest et al. (1987) found that female DRL performance was impaired when an object is placed inside the operant conditioning chamber with her during testing. Male performance on the DRL task, however, was not impaired by the presence of an object. This finding suggests that females are more easily distracted by the object and performance on the task suffers. Therefore, an inhibitory control task that encompasses an attentional component would further elucidate the differences in male and female behavior and performance in an inhibitory control task.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Long Evans rats were perinatally exposed to 0, 3, or 6 mg/kg/day PCBs.
- One adult male and female/litter were tested on an operant inhibitory control task following microinjection of one of three different dopaminergic agonists into the medial prefrontal cortex.
- Four doses each of bupropion, SKF81297, and quinpirole were used (n=6–9 male-female littermate pairs/exposure group/drug).
- Increasing doses of all three dopaminergic agonists provided a performance benefit with no unique therapeutic benefit to PCB-exposed animals.

Figure 1.

Figure 1a (top): Reinforced:Nonreinforced lever presses collapsed across testing block. There was a significant sex x PCB exposure group interaction, wherein control females had a significantly higher ratio than control males ($^A p < .05$) and 3mg/kg/day females ($^* p < .05$). Figure 1b (bottom). Number of reinforcers earned by sex and exposure group across all blocks. There was significant sex x exposure group interaction on number of reinforcers earned wherein control females earned significantly more reinforcers than control males ($^A p = .036$) as well as the 3 mg/kg/day females ($^* p = .014$).

Figure 2.

Reinforced:nonreinforced lever presses collapsed across exposure group. A significant sex x block interaction revealed that females had a significantly higher ratio of reinforced:nonreinforced lever presses than males for blocks 5 – 7 (* $p < .05$).

Figure 3.

IRT analysis collapsed across sex and block. A significant IRT bin x exposure group interaction was found wherein the 3 mg/kg/day PCB-exposed rats exhibited a significantly higher proportion of responses in the 0.0–2.49 s time bin and a significantly lower proportion of responses in the 15.0–17.49 s time bin compared to control rats (* $p < .05$).

Figure 4.

IRT analysis by sex and exposure group. Control (0 mg/kg/day PCB) males had a higher proportion of responses in the 5.0–7.49 s through 12.5–14.99 s ($p = .001$) time bins as well as a lower proportion of responses in the 15.0–17.49 s and the > 17.5 s time bins compared to control (0 mg/kg/day PCB) females while males from the 6 mg/kg/day group had a higher proportion of responses in the 12.5–14.99 s time bin and a lower proportion of responses in the > 17.5 s compared to 6 mg/kg/day females ($^A p < .05$ versus same exposure group females). In addition, control males also had a lower proportion of responses in the 0.0–2.49 s time bin and a higher proportion of responses in the 12.5–14.99 s time bin compared to the 3 mg/kg/day males while control females had a higher proportions of responses in the > 17.5 s time bin compared to the 3 mg/kg/day females ($*p < .05$ versus same sex controls).

Figure 5.

Effect of bupropion doses on number of reinforcers earned. There was a significant dose x exposure group interaction on the number of reinforcers earned wherein bupropion led to a significant increase in the number of reinforcers earned for the control animals (7 male/female pairs) at the .01 μg/side and .12 μg/side doses compared to the 0 μg/side dose. Likewise, rats exposed to 6 mg/kg/day (8 male/female pairs), but not the 3 mg/kg/day (9 male/female pairs), also showed improvement after receiving the .06 and .12 μg/side dose compared to the 0 μg/side dose (* $p < .05$).

SKF81297 Reinforcers Collapsed Across Sex

Figure 6.

There was a significant main effect of SKF81297 dose, $F(3, 60) = 30.564$, $p < .001$, as well as a significant main effect of sex, $F(1, 20) = 6.310$, $p = .021$, on the number of reinforcers earned. However, as shown in the figure, no PCB-related effects were found with this agonist.

Figure 7.

Sex x dose x exposure group interaction on the number of reinforcers earned, $F(6, 60) = 2.971$, $p = .013$. For control males ($n = 6$): the increase in quinpirole dose from 0 μg/side to .0.1 μg/side ($p = .016$) and from 0 μg/side to 1.0 μg/side ($p = .031$). For 3 mg/kg/day males ($n = 9$): the increase from 0 μg/side to .0.1 μg/side ($p = .005$), from 0 μg/side to 0.5 μg/side ($p = .009$), and from 0 μg/side to 1.0 μg/side ($p < .001$). For 6 mg/kg/day males ($n = 8$): the increase from 0 μg/side to .0.1 μg/side ($p = .046$), from 0 μg/side to 0.5 μg/side ($p = .041$), and from 0 μg/side to 1.0 μg/side ($p = .013$). For control females ($n = 6$): the increase from 0 μg/side to 0.5 μg/side ($p = .034$) and from 0 μg/side to 1.0 μg/side ($p = .008$). For 3

mg/kg/day females ($n = 9$): the increase from 0 $\mu\text{g}/\text{side}$ to 0.5 $\mu\text{g}/\text{side}$ ($p = .009$) and from 0 $\mu\text{g}/\text{side}$ to 1.0 $\mu\text{g}/\text{side}$ ($p = .028$). For the 6 mg/kg/day females there was no effect of quinpirole dose on the number of reinforcers earned.

Table 1

Dependent Variables to be used in Data Analyses

Type of Measure	Dependent variables
Physiological	
From Dam	<ul style="list-style-type: none"> a. Liver weight b. Implantation sites c. Percent gestational weight gain d. Percent lactational weight gain
From Pups	<ul style="list-style-type: none"> a. Postnatal weight gain: PND 0, 7, 14, 21 b. Organ weight analysis (one male and female/litter) c. Brain weight:body Weight d. Liver weight:body Weight e. Thymus weight:body Weight
Behavioral	
Baseline DRL 15 [*]	<ul style="list-style-type: none"> a. Reinforced:non-reinforced Responses b. Reinforcers earned c. Proportion of responses within each 2.5 IRT Bin
DA Agonist Drug Challenges	<ul style="list-style-type: none"> a. Reinforced:non-reinforced responses b. Reinforcers earned c. Proportion of responses within each 2.5 IRT bin

Note.

* Presented in 5 day blocks

TABLE 2

Reproductive Outcomes of Dams Exposed to 0, 3, or 6 mg/kg/day PCB (Fox River Mix) during Gestation and Lactation

PCB Dose	Dam Gest. Weight Gain (g)	Dam Lact. Weight Gain (g)	Dam Liver:Body Weight Ratio
0 mg/kg/day (18 litters)	42.21 ± 1.92	9.42 ± 1.60	.05522 ± .0016
3 mg/kg/day (21 litters)	43.50 ± 1.48	9.63 ± 1.14	.05531 ± .0019
6 mg/kg/day (20 litters)	44.91 ± 1.78	9.95 ± 1.01	.05286 ± .0016

Note. Weights are in grams; Means (± SEM)

TABLE 3

Average Body Weight ($\pm$ SEM) for Pups Born to Dams Exposed to 0, 3, or 6 mg/kg/day PCB (Fox River Mix) during Gestation and Lactation

	PND 0	PND 7	PND 14	PND 21
0 mg/kg/day PCB (18 litters)				
MALE	5.90 $\pm$ 0.11	13.56 $\pm$ 0.36	27.65 $\pm$ 0.60	43.25 $\pm$ 1.07
FEMALE	5.63 $\pm$ 0.09	13.14 $\pm$ 0.32	26.91 $\pm$ 0.53	41.68 $\pm$ 1.03
3 mg/kg/day PCB (21 litters)				
MALE	5.69 $\pm$ 0.12	12.39 $\pm$ 0.34	25.14 $\pm$ 0.56	37.95 $\pm$ 1.01

FEMALE	5.52 $\pm$ 0.09	11.84 $\pm$ 0.36	24.44 $\pm$ 0.51	35.07 $\pm$ 1.80
6 mg/kg/day PCB (20 litters)				
MALE	5.84 $\pm$ 0.09	11.86 $\pm$ 0.23	23.75 $\pm$ 0.53	35.69 $\pm$ 1.03

FEMALE	5.48 $\pm$ 0.08	11.32 $\pm$ 0.24	22.77 $\pm$ 0.46	33.81 $\pm$ 0.83

Note. Body weights are in grams;

* $p < .05$

** $p < 0.01$

*** $p < 0.001$ from the same-sex 0 mg/kg/day PCB dose

TABLE 4

Developmental Mortality Data for Pups Born to Dams Exposed to 0, 3, or 6 mg/kg/day PCB (Fox River Mix) during Gestation and Lactation

Birth	Litter Size	%Males	%Live	%Mortality
0 mg/kg/day PCB (18 litters)	12.72	46.020	100.00	4.260
3 mg/kg/day PCB (21 litters)	12.71	45.160	93.323	9.358
6 mg/kg/day PCB (20 litters)	12.15	47.568	97.929	9.547

Note: All data presented in table are means

TABLE 5Average ($\pm$ SEM) Organ Weight Analysis on PND 21 for Pups Exposed to the Fox River PCB Mixture During Gestation and Lactation

PCB Exposure Group	Brain Weight	Liver Weight	Thymus Weight
0 mg/kg/day (18 litters)			
MALE	1.440 $\pm$ 0.021	1.723 $\pm$ 0.106	0.186 $\pm$ 0.008
FEMALE	1.411 $\pm$ 0.018	1.648 $\pm$ 0.103	0.187 $\pm$ 0.010
3.0 mg/kg/day (21 litters)			
MALE	1.443 $\pm$ 0.019	2.612 $\pm$ 0.098	0.133 $\pm$ 0.008
		}	

FEMALE	1.382 $\pm$ 0.017	2.389 $\pm$ 0.096	0.140 $\pm$ 0.009
6.0 mg/kg/day (20 litters)			
MALE	1.385 $\pm$ 0.020	2.409 $\pm$ 0.101	0.114 $\pm$ 0.008
		}	

FEMALE	1.339 $\pm$ 0.017	2.206 $\pm$ 0.098	0.101 $\pm$ 0.009

Note. Weights are in grams;

**

 $p < 0.01$.

 $p < 0.001$

TABLE 6

Average ($\pm$ SEM) Organ: Body Weight Ratios on PND 21 for Pups Exposed to the Fox River PCB Mixture During Gestation and Lactation

PCB Exposure Group	Brain:body weight ratio	Liver:body weight ratio	Thymus:body weight ratio
0 mg/kg/day (18 litters)			
MALE	0.0338 ± 0.0008	0.0400 ± 0.0011	0.0043 ± 0.0002
FEMALE	0.0344 ± 0.0008	0.0400 ± 0.0011	0.0045 ± 0.0002
3.0 mg/kg/day (21 litters)			
MALE	0.0382 ± 0.0011	0.0682 ± 0.0015	0.0034 ± 0.0001
		}	}
		**	***
FEMALE	0.0393 ± 0.0015	0.0665 ± 0.0022	0.0038 ± 0.0002
6.0 mg/kg/day (20 litters)			
MALE	0.0401 ± 0.0012	0.0677 ± 0.0020	0.0032 ± 0.0002
		}	}
		**	***
FEMALE	0.0407 ± 0.0010	0.0657 ± 0.0022	0.0030 ± 0.0001

Note. Weights are in grams;

**
 $p < 0.01$.***
 $p < 0.001$