Gender dependent and genotype sensitive dopaminergic changes induced by polychlorinated biphenyl 153 in the rat brain.

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Abbrevations: 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, serotonin; DA, dopamine; DAT, dopamine transporter; DR, dopamine receptor; DHBA, 3, 4-hydroxybenzylamine; GABA, γ-amino butyric acid; Gln, glutamine; Glu, glutamate; Gly, glycine; HPLC, high performance liquid chromatography; HVA, homovanillic acid; PCB, polychlorinated biphenyl; p, postnatal day; Ser, serine; SERT, serotonin transporter; SHR, spontaneously hypertensive rats; Tau, taurine; TH, tyrosine hydroxylase; V-MAT2, vesicular monoamine transporter-2; WKY, Wistar Kyoto

Abstract

Polychlorinated biphenyls (PCBs) can be separated into *ortho*- and non-*ortho*-substituted PCBs, where most of the *ortho*-substituted being neurotoxic and possibly interrupt cognitive functions. The present study examined the effect of the *ortho*-substituted PCB 153 on dopamine and serotonin neurotransmitters as well as amino acids in the neostriatum of both genders from the Wistar Kyoto (WKY) and the spontaneously hypertensive rat (SHR) genotypes. Exposure to PCB 153 led to increases in homovanillic acid and 5-hydroxyindoleacetic acid, the degradation products of dopamine and serotonin, respectively, in all groups except for the female SHRs, whereas levels of dopamine and serotonin neurotransmitters as well as amino acids were unchanged in all genotypes and genders. PCB-153 also induced a decrease in the neostriatal D5 receptor in both genders and genotypes, without changing the D1 receptor. In contrast, levels of the dopamine transporter were reduced in the WKY male, together with an insignificant reduction of the mean in the SHR male. In addition, a gender-specific decrease of the PSD-95 protein occurred in the PCB-exposed male rats. Levels of tyrosine hydroxylase and vesicular monoamine transporter-2 were unchanged in all animals examined. Therefore, postnatal PCB exposure had major effects on both dopamine and serotonin turnover as well as specific PCB-sensitive synaptic proteins. Differences occurred between the effects obtained in both genotypes, as well as between genders.

Introduction

Polychlorinated biphenyls (PCBs), persistent industrial contaminants are widely distributed in the environment (Kodavanti & Tilson, 1997;Mariussen & Fonnum, 2006). They can be separated into *ortho*- and non-*ortho*-substituted PCBs, with most of the *ortho*-substituted being neurotoxic. These lipophilic compounds enter the human body both prenatally through the placenta and postnatally through breast milk and fatty food (Safe, 1994). PCBs may be associated with behavioural and cognitive dysfunctions in both humans, non-human primates and rodents (Chevrier *et al.*, 2008;Johansen *et al.*, 2011;Johansen *et al.*, 2014;Schantz *et al.*, 2003).

Underlying biological mechanisms of PCBs may include changes in neurotransmission and synaptic plasticity, Ca^{2+}- dynamics and oxidative stress responses (Mariussen & Fonnum, 2006;Seegal *et al.*, 2010). In particular, several PCBs may induce strong inhibition of neurotransmitter transporters, including the Na^{+}-dependent plasma membrane dopamine transporter (DAT) and the H^{+}-dependent vesicle monoamine transporter-2 (VMAT-2) (Mariussen & Fonnum, 2001;Seegal *et al.*, 2010;Wigestrand *et al.*, 2013). Such effects may change interactions between e.g., monoaminergic and amino acidergic transmitter systems, specifically those mediated by neurons employing dopamine (DA) or serotonin (5-HT) and those using glutamate (Glu) as transmitters (Arnsten *et al.*, 2012;Lisman & Grace, 2005). This kind of interaction may be linked to a number of behavioural and psychiatric dysfunctions, including Attention Deficit Hyperactivity Disorder (ADHD). This condition is characterized by hyperactivity, impulsiveness and inattention, and usually occurs with onset in early childhood but may also persist into adulthood (Biederman & Faraone, 2005). The PCBs represent one type of environmental factor that either alone or in concert with a genetic vulnerability may contribute to the prevalence of ADHD (Williams & Ross, 2007;Mariussen & Fonnum, 2001;Holene *et al.*, 1998).

We have recently reported the effects of PCB 153 on the behaviour of spontaneously hypertensive rats (SHRs) obtained from Charles River and in the Wistar Kyoto (WKY) strain obtained from Harlan, UK (Johansen *et al.*, 2011;Johansen *et al.*, 2014). At the present time, the SHR rats appears to constitute the best validated animal model of ADHD, combined subtype (ADHD-C) (Sagvolden & Johansen, 2012). Our data

indicate that postnatal exposure to PCB 153 led these strains, which are genetically related (DasBanerjee *et al.*, 2008), to develop distinct behavioural changes, as determined by an operant procedure which measured sustained attention, hyperactivity and impulsivity. Both strains also showed gender differences in behaviour (Johansen *et al.*, 2014).

We therefore found it of interest to investigate whether such PCB-153 exposure also led to differences in monoamine or amino acid transmitter dynamics in the brains. For this purpose, we employed HPLC analysis of extracts from the neostriata in order to determine levels of neurotransmitters and their metabolites in a way which allowed us to calculate turnover ratios for, e.g., the monoamines dopamine (DA) and serotonin (5HT) as well as the amino acid transmitter glutamate (Glu). Finally, we also performed a detailed immunochemical analysis of the nigrostriatal DA synapses in the samples from PCB 153-exposed and control animals. Our data indicate that PCB 153 exposure induced major enhancements of DA and 5HT turnover in all male animals, whereas in the female, only the WKY responded. Moreover, the levels of a distinct set of proteins present in dopaminergic synapses were affected, one of which responded in both sexes, while others responded specifically either in male or in female rats.

Methods

Animals and PCB exposure

Wistar Kyoto (WKY/NHsd) and spontaneously hypertensive (SHR/NCrl) rats were bred at the Norwegian Defense Research Establishment (Kjeller, Norway). The dams were kept under standard laboratory animal conditions (22°C, 55% humidity and 12 hr light/dark cycle) in type IV macrolon cages and aspen bedding, with free access to food (RM (E), Special Diet Services, Witham Essex, UK) and water. Offsprings of both genders were exposed to corn oil without or with either 6 mg PCB 153 (2,2`, 4,4`, 5,5`-hexachlorobiphenyl) per kg body weight during the lactational period. PCB 153 dissolved in corn oil was given through an orally inserted stomach tube. To avoid acute toxicity, the compound was administered at postnatal day p 8, p14 and p20, in a total volume of 0.01 ml/g body weight for each exposure. At p55-60, rats treated with 6 mg/ kg body weight of PCB 153 and a set of age-matched control rats were decapitated and the neostriata were dissected and subsequently prepared for neurotransmitter analysis as previously described (Dervola *et al.*, 2012). PCB was administered through multiple doses to avoid acute toxicity, and was given during the lactational period because this period has been reported to have a more profound PCB-induced impact on gene expression than, e.g., during the gestational period (Sazonova *et al.*, 2011). Procedures and experiments were conducted in accordance with Norwegian laws and regulations, and were approved by the Norwegian Animal Research Authority (NARA). The PCB 153 preparation used in this study was specially purified and free from dioxine-like PCBs (a gift from Dr. Patrik Anderson, University of Umeå, Sweden).

Sample collection. At p55-60, both control and PCB-treated WKY and SHR rats of both genders were stunned and rapidly decapitated. The neostriata were removed, frozen in liquid N_2, and stored at -70°C for later sample preparation and analyses.

Immunoblotting of striatal proteins

Preparation of striatal proteins. One neostriatum from each animal was homogenized in a glass/Teflon Potter-Elvehjem homogenizer by hand in 30 strokes, with 200 µl of a buffer containing 1 mM EDTA, 10 mM Hepes and 1x

proteaseinhibitor (Complete coctail from Roche). Half of the homogenate was transferred to HPLC analysis, while the other half was destined for western analysis and was added to a buffer containing 1 mM EDTA, 10 mM Hepes, 50 mM NaCl, 1 % SDS and 1x proteaseinhibitor cocktail (Roche), homogenates were stored at -70°C until use. Solutions were made with purified 18Ω/cm H_2O (Milli-Q Advantage A10, Millipore).

Immunoblotting analysis. The analysis was performed as described in (Bogen *et al.*, 2006). Antibodies used for immunoblotting studies included rabbit primary antibodies against TH AB151 (diluted 1: 20 000 in 5% BSA, secondary antibody contained 5% dry-milk) from Chemicon International (Billerica, United States), DAT (H-80) SC14002 (diluted 1: 2000 in 0.5% BSA) from Santa Cruz (Heidelberg, Germany), PSD-95 (SAP-90, DLG 4) 124 002 (diluted 1: 6000 in 0.5% BSA) from Synaptic Systems (Göttingen, Germany), D1 receptor SC14001 (diluted 1: 300 in 1% BSA, secondary antibody contained 0.5% dry-milk) from Santa Cruz (Heidelberg, Germany), D5 receptor 20310-1-AP (diluted 1: 4000 in 0.5% BSA) from Proteintech (Manchester, United Kingdom), and VMAT-2 138302 (diluted 1.5: 10 000 in 1% BSA) from Synaptic Systems (Göttingen, Germany). The mouse primary antibodies included Tuj1 MMS-435-P (diluted 1: 40 000 in 2.5% dry-milk, secondary antibody contained 1.25% dry-milk) from Nordic Biosite (Oslo, Norway), Na^+/K^+-ATPase ab7671 (diluted 1: 5000 in 0.5% BSA) from Abcam (Cambridge, United Kingdom), and β-actin (diluted 1: 750 000 in 0.5% BSA) from Sigma Aldrich (St. Louis, MA, USA). Secondary anti-rabbit Ig horseradish peroxidase and anti-mouse Ig horseradish peroxidase were obtained from Amersham Biosciences (Buckinghamshire, UK), and both were diluted 1:10 000 with presence of either BSA or dry-milk as noted above, before incubation. Testing of the different antibodies included dilution curves with 2.5, 5, 7.5 and 10 µg neostriatal proteins.

Biochemical neurotransmitter analysis of striatal tissue

Chemicals. Monoamine and amino acid analyses were done with high performance liquid chromatography (HPLC). L-amino acid standards, including aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glutamine (Gln) and glycine (Gly) were obtained from Pierce (Rockford, Ill., USA). Taurine (Tau), γ-amino butyric acid (GABA), and α-

amino adipic acid as well as the monoamine standards dopamine (DA), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA) and 3, 4-hydroxybenzylamine (DHBA), as well as $HClO_4$ and ascorbic acid were from Sigma-Aldrich (Steinheim, Germany). The BCA-assay kit (Thermo-Scientific, Rockford, USA), and n-hexane (Merck, Darmstadt, Germany) were from VWR. Solutions were made with purified 18MΩ/cm H_2O (Milli-Q Advantage A10, Millipore).

Extract preparation and protein assay. Homogenates for HPLC-analysis were prepared as described above. Samples were thawed, and aliquots were mixed with ice cold $HClO_4$ (0.2 M) to precipitate proteins. This suspension was mixed with an equal volume of DHBA (used as internal standard for monoamines) and in 0.12 mM ascorbic acid to a final concentration of 0.227 µM DHBA. The homogenates were centrifuged for 20 min at 15000 x g at 2°C in a Sorvall RMC-14-microcentrifuge and the pellets were frozen at -40°C. Protein determination was performed by dissolving pellets in 0.1 M NaOH and measuring protein content by the BCA assay (Smith *et al.*, 1985). The supernatants were extracted with equal volumes of n-hexane to reduce lipid contamination, and the top layer was discharged. For amino acid analyses, parts of the delipidized $HClO_4$ extracts were mixed with a solution of α-amino adipic acid (used as internal standard) to a final concentration of 30 µM in a total volume of 1.9 mL. These extracts were neutralized to pH 7.2 with ice cold KOH and centrifuged for 20 min at 15000 x g at 2°C as described above. Supernatants were stored at -70°C until analyses. The samples were then thawed, and transferred into glass-vials through Nylon-66 micro filters (0.22 µm; Nalgene, Rochester, NY, USA), prior to HPLC analysis.

Monoamine analysis. Column and mobile phases, selected for analysis of catecholamine content in plasma, were supplied by Chromsystems (Germany). The frozen extracts were used directly for analyses of total DA and 5-HT, as well as their metabolites HVA and 5-HIAA, using a reversed-phase HPLC (Shimadzu, Kyoto, Japan) with electrochemical detection (ECD; Decade II with Sencell flow electrode, Antec Leyden) at a working potential of 0.6 V. Each sample was eluted for 40 min with a flow rate at 1.3 mL/min. External standard solutions of DA, HVA, 5-HT, 5-HIAA and DHBA were analysed on the same day. The chromatograms were analysed using the software Lab Solutions (Shimadzu, Kyoto, Japan). Monoamine concentrations are presented in pmol/mg total neostriatal protein.

Amino acid analysis. Using a Chromspher 5 C18 column of 25 cm length and 4.6 mm inner diameter (Varian), total amino acids in the neostriatum extracts were analysed (Hassel *et al.*, 1997) using reversed-phase HPLC fitted with a fluorescence detector (Shimadzu) after derivatization with o-phthaldialdehyde (OPA; Sigma). The mobile phase comprised 75% 50 mM phosphate buffer (pH = 5.25), and 25% methanol (v/v), changing linearly to 25% phosphate buffer and 75% methanol during 26.5 min, after which the methanol concentration was linearly reduced to 15%. Each sample was eluted for 45 min with a flow rate at 0.4 mL/min. A mixture of the amino acids of interest, all at a concentration of 100 µM, was used as external standards. The chromatograms were analysed using the Lab Solutions software. Amino acid concentrations are expressed in nmol/mg total neostriatal protein.

Statistical analysis. Statistical significance of differences between biochemical samples was determined by unpaired, two-tailed Student's *t*-test, where $p < 0.05$ was defined as significant. Data given as mean ± SEM, were tested for normal distribution and unequal variance. Welch`s correction was used when there was unequal variance. Analysis was performed with GraphPad Prism.

Results

PCB 153-induced increased neostriatal turn-over rate of DA and 5-HT.
We decided to examine the effects of PCB-153 because this congener is the one with highest concentration in human milk and has a slow turn over in human tissue (Seegal *et al.*, 2011). Moreover, the PCB was administrated in multiple doses to avoid acute toxicity, and given during the lactational period which is more sensitive than the gestational period, in order to affect gene expression (Sazonova *et al.*, 2011). Using this approach, we first elucidated genotypic and gender-specific effects in neostriatal dopaminergic and serotonergic systems induced by postnatal PCB 153 exposure. For this purpose we measured dopamine (DA) and serotonin (5-HT) and their respective degradation products, homovanillic acid (HVA) and 5-hydroxyindole-3-acetic acid (5-HIAA), in neostriata dissected from both genders of WKY and SHR rats (Figure 1A).

DA and HVA. Administration of PCB 153 did not change the levels of DA (Fig 1A), but induced major changes in HVA (Fig 1B) and consequently the calculated turnover of DA (HVA/DA) in both WKY and SHR rats (Fig 1C) (Huotari *et al.*, 2002;Jones *et al.*, 1986). In contrast, the level of HVA and the calculated turnover of DA were insignificantly changed by PCB 153 in the female SHR.

5-HT and 5-HIAA. Similar to the DA response, the 5-HT levels (Fig 1D) were unaffected in both the genotypes and genders after PCB exposure. However, the 5-HT levels appeared to be decreased in the female SHRs of both the untreated and PCB-treated group compared to levels found in the untreated male SHRs, indicating a possible gender difference between the 5-HT, but not the DA systems in the SHRs. Analysis of the metabolite 5-HIAA (Fig 1E) showed a significant increase induced by PCB153-exposure in both genders of the WKY and the male SHR, whereas 5-HIAA in the female SHR was unchanged. These effects led to the calculated turnover of 5-HT (Fig 1F) being increased in both genders of the WKY as well as the male SHR, and reaching a similar level after the PCB treatment in these animals. In contrast, due to the low levels of both 5-HT and 5-HIAA in the female SHRs, the calculated turnover of 5HT was already high in this animal and it remained unchanged by PCB 153 treatment.

Amino acids. There were no significant changes in the amino acid levels between the genotypes or after exposure to PCB 153 (Table 1).

Effects of PCB 153 on presynaptic dopaminergic markers

PCB exposure led to a significant 30% reduction in the DAT levels (Table 2), which was restricted to the male WKY. A similar change in the mean value was found in the PCB exposed male SHR, but due to a larger variance (DasBanerjee *et al.*, 2008), this decrease failed to reach a significant p-value. PCB-exposure had no effect on DAT in the females.

VMAT-2 levels (Table 2) appeared to be similar in the untreated controls of both genotypes, and PCB-exposure did not induce significant VMAT-2 changes in any of the male animals or in the female WKYs. However, in the female SHRs, a tendency of PCB-induced enhancement was seen (~40%, p=0.0556).

PCB-exposure had no effects on the levels of TH (Table 2), the rate-limiting enzyme in DA synthesis, in either gender of the WKY or SHR rats.

Effects of PCB 153 on postsynaptic dopaminergic markers

Following PCP-153 exposure, no differences were seen in the D1 receptor in any of the rats, whereas a significant reduction in the D5 receptor was seen in both genotypes and genders (Table 2). This interesting difference between the D1 and D5 DA receptors appears to be a novel observation.

Analysis of the postsynaptic marker protein PSD-95 (Table 2), which is present in both glutamatergic and DA synapses (El-Husseini *et al.*, 2000;Kennedy, 1998;Zhang *et al.*, 2007), showed no differences in the untreated animals between either genotype or gender. In the PCB treated animals, a reduction was seen in the male gender only.

Neither the neurospecific tubulin isoform TUJ-1 (Lee *et al.*, 1990) nor the general plasma membrane marker Na^+/K^+-ATPase were changed following PCB-153 exposure in any of the rats (Table 2).

DISCUSSION

In the present study, we have examined whether exposing young rats to the PCB 153-congener led to changes in monoaminergic and amino acidergic neurotransmission in the neostriata of WKY and SHR rats. Addition of PCB 153 had no effect on the levels of total DA in the young adults examined. Other studies have found decreased amount of total DA and 5-HT, although this was measured as an acute effect after an extremely high single dose of a PCB mixture (Seegal *et al.*, 1986a;Richardson & Miller, 2004;Seegal *et al.*, 1986b). The approach used here allowed us to observe that the turnover ratios of both DA and 5-HT represented as sensitive markers of PCB exposure and they were enhanced following exposure to PCB 153 in both male and female WKY, as well as in male SHR, but not in the female SHRs. No effects were seen in the measured amino acid transmitters. We also found an effect of PCB 153 on DAergic proteins and in addition, for the first time an effect on the D5 receptors.

DA and 5-HT analysis

We compared the effects of PCB 153 on the DA system in more detail by exploiting the dense nigrostriatal DA innervation of neostriatum, where all striatal DA is present in nerve terminals. Initially, we determined the levels of DA and 5-HT in PCB-treated animals, and observed that no changes under the experimental conditions. These data indicate that PCB-exposure had minor effects on the metabolism of these monoamine transmitters. However, this conclusion was not consistent with our analysis of the DA and 5-HT metabolites, HVA and 5-HIAA, which rather indicated that PCB 153 induced major increases in neurotransmitter metabolism. Both HVA and 5-HIAA levels as well as the HVA/DA and 5-HT/5-HIAA ratios were increased by 3-4 fold in both of the WKY genders and in male SHRs, although not in the female SHRs. This indicates increased levels of extracellular DA and 5-HT, respectively (Jones et al. 1998). This finding is consistent with a previous research report showing that PCB-exposure may lead to increased extracellular DA, without changing the general tissue concentration (Seegal *et al.*, 2002).

Although studies on the 5-HT system are limited, different PCB congeners have been reported to effect the 5-HIAA levels, but this has been performed with higher doses and in different brain regions and different exposure procedure (Boix & Cauli, 2012). Well-defined ascending serotonergic raphestriatal fibers have been described (Miller *et al.*, 1975). Our results clearly demonstrate that PCB 153-exposure induces a strong 5-HT turnover increase in these fibers similar to that observed in the DA synapses. This makes it possible that PCB 153 may have similar effects on the raphestriatal 5-HT synapses as those occurring in the nigrostriatal DA synapses.

Changes such as increased DA and 5-HT metabolites could potentially be caused by more frequently evoked transmitter release, DAT or SERT inhibition, or upregulation of reverse DAT and SERT transport mechanisms. Also, the activity and amount of COMT or ALDH, the enzymes responsible for synthetizing HVA and 5-HIAA, respectively (Some & Helander, 2002;Huotari *et al.*, 2002), may be of importance for the enhanced amounts of metabolites observed. Recent studies have indicated that exposure of rats to PCB mixtures may enhance expression of the COMT gene (Sazonova *et al.*, 2011), but to what extent ALDH is sensitive to PCBs appears to be relevant for further exploration.

Therefore, the stable DA and 5-HT transmitter levels seen in the PCB-exposed animals do not necessarily indicate stable metabolism, but may rather indicate that the terminals undergo compensatory adjustments.

Presynaptic markers

Immunochemical approaches were used to examine molecular changes in the DA synapse by quantitating DAT, which is a Na^+-dependent plasmalemmal transporter responsible for translocating the extracellular DA back into the neuronal cytoplasm, as well as TH, the rate-limiting enzyme in DA synthesis and VMAT-2, the proton-dependent vesicular monoamine transporter determining DA storage efficiency in the vesicles.

Comparing these proteins, the level of DAT was significantly decreased in the male WKY. Furthermore, a similar effect in the mean value of DAT was also seen in the male SHR, but statistical analysis showed that experimental variation in this genotype

was higher, precluding statistical significance. In the females, no changes in DAT levels were seen. Another study has also found decreased DAT, although this was measured as an acute effect after a very high single dose of a PCB mixture (Richardson & Miller, 2004). An inverse relationship between DAT and PCB levels has also been demonstrated in human females but not in males, showing that the DAT may be hormone sensitive (Seegal *et al.*, 2010). The DAT transporter protein is subject to complex trafficking and posttranslational modifications, some of which are substrate-dependent (Zahniser & Sorkin, 2009). DAT may therefore be importantly involved in the pathophysiology of, e.g., ADHD (Bowton *et al.*, 2014;Mergy *et al.*, 2014). Moreover, DAT activity is known to be decreased following direct binding to PCB congeners *in vitro* (Mariussen & Fonnum, 2001;Wigestrand *et al.*, 2013). In contrast, DAT were insensitive to PCB 153 in the female rats, although the WKY females (but not the SHR) still had a PCB 153-induced increase in HVA. Recent studies have indicated that Aroclor exposure may enhance expression of the COMT gene (Sazonova *et al.*, 2011), while other reports have indicated that the DAT gene is involved in gender-specific hormonal-dependent behavioral effects (Fossella *et al.*, 2002). To what extent such mechanisms may be involved in the regulation of HVA levels after PCB153 exposure in the female WKY and SHR rats, remains to be determined.

Levels of VMAT-2 were not sensitive to PCB in the male and female WKY, but in the female SHRs, a tendency of an increase was seen. Another study has demonstrated PCB-induced decrease of VMAT-2, although their PCB concentration was much higher, and the effect was measured acutely (Richardson & Miller, 2004).

Finally, the levels of TH were completely resistant to PCB 153 in all animals under the present conditions. In agreement with these data, researchers (Kodavanti *et al.*, 1998;Richardson & Miller, 2004) have exposed rats to high doses of PCB mixtures and found no changes in TH (Richardson & Miller, 2004).

Postsynaptic mechanisms

In addition to these presynaptic effects, we also examined possible PCB 153 effects on the postsynaptic dopamine D1/5 receptors. In the neostriatum, the D1 receptor is

predominantly present in the direct medium spiny neurons (dMSN) which represent ~ 45% of total cells in the neostriatum (Bolam *et al.*, 2000;Gerfen *et al.*, 1990;Surmeier *et al.*, 2011). Our data demonstrate that these D1 receptors are insensitive to PCB 153 exposure, making it likely that the dMSNs may survive the PCB treatment. In agreement, previous work by (Roth-Harer *et al.*, 2001) did not find any effect on the two main dopamine receptors the D1 and D2 receptor after exposure to PCB 77. In contrast, we found a consistent PCB-induced decrease of the D5 receptors (~ 40%) was found in both genotypes and genders. The D5 isoform is present in a number of interneurons in the striatum, including the giant cholinergic interneurons as well as several GABAergic interneurons which directly innervate the MSN population (Surmeier *et al.*, 2011). Interestingly, many of these interneurons are autonomous pacemakers (Ding *et al.*, 2010;Tepper *et al.*, 2010). Hence, PCB effects in the neostriatum mediated by loss of D5 receptors may occur through interneurons, many of which modulate spontaneous action potential firing.

Conclusion

In conclusion, we have discovered that postnatal PCB exposure prior to weaning has major and similar effects on DA and 5HT turnover in the neostriatum of the young adult rats, without changing levels of the transmitters themselves. The dopaminergic and serotonergic systems may therefore be subject to essentially identical regulations. Detailed analysis of DA-synapses demonstrated specific effects on a restricted number of specific synaptic proteins, including the presynaptic DAT transporter and the postsynaptic D5 receptor. Clear differences were also seen between the distinct genotypes (WKY and SHR), as well as between male and female genders.

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Reference List

Arnsten AF, Wang MJ, & Paspalas CD (2012). Neuromodulation of thought: flexibilities and vulnerabilities in prefrontal cortical network synapses. *Neuron* **76**, 223-239.

Biederman J & Faraone SV (2005). Attention-deficit hyperactivity disorder. *Lancet* **366**, 237-248.

Bogen IL, Boulland JL, Mariussen E, Wright MS, Fonnum F, Kao HT, & Walaas SI (2006). Absence of synapsin I and II is accompanied by decreases in vesicular transport of specific neurotransmitters. *J Neurochem* **96**, 1458-1466.

Boix J & Cauli O (2012). Alteration of serotonin system by polychlorinated biphenyls exposure. *Neurochem Int* **60**, 809-816.

Bolam JP, Hanley JJ, Booth PA, & Bevan MD (2000). Synaptic organisation of the basal ganglia. *J Anat* **196 (Pt 4)**, 527-542.

Bowton E, Saunders C, Reddy IA, Campbell NG, Hamilton PJ, Henry LK, Coon H, Sakrikar D, Veenstra-VanderWeele JM, Blakely RD, Sutcliffe J, Matthies HJ, Erreger K, & Galli A (2014). SLC6A3 coding variant Ala559Val found in two autism probands alters dopamine transporter function and trafficking. *Transl Psychiatry* **4**, e464.

Chevrier J, Eskenazi B, Holland N, Bradman A, & Barr DB (2008). Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *Am J Epidemiol* **168**, 298-310.

DasBanerjee T, Middleton FA, Berger DF, Lombardo JP, Sagvolden T, & Faraone SV (2008). A comparison of molecular alterations in environmental and genetic rat models of ADHD: a pilot study. *Am J Med Genet B Neuropsychiatr Genet* **147B**, 1554-1563.

Dervola KS, Roberg BA, Woien G, Bogen IL, Sandvik TH, Sagvolden T, Drevon CA, Johansen EB, & Walaas SI (2012). Marine Omega-3 polyunsaturated fatty acids induce sex-specific changes in reinforcer-controlled behaviour and neurotransmitter metabolism in a spontaneously hypertensive rat model of ADHD. *Behav Brain Funct* **8**, 56.

Ding JB, Guzman JN, Peterson JD, Goldberg JA, & Surmeier DJ (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* **67**, 294-307.

El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, & Bredt DS (2000). PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364-1368.

Fossella J, Sommer T, Fan J, Wu Y, Swanson JM, Pfaff DW, & Posner MI (2002). Assessing the molecular genetics of attention networks. *BMC Neurosci* **3**, 14.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., & Sibley DR (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**, 1429-1432.

Hassel B, Bachelard H, Jones P, Fonnum F, & Sonnewald U (1997). Trafficking of amino acids between neurons and glia in vivo. Effects of inhibition of glial metabolism by fluoroacetate. *J Cereb Blood Flow Metab* **17**, 1230-1238.

Holene E, Nafstad I, Skaare JU, & Sagvolden T (1998). Behavioural hyperactivity in rats following postnatal exposure to sub-toxic doses of polychlorinated biphenyl congeners 153 and 126. *Behav Brain Res* **94**, 213-224.

Huotari M, Gogos JA, Karayiorgou M, Koponen O, Forsberg M, Raasmaja A, Hyttinen J, & Mannisto PT (2002). Brain catecholamine metabolism in catechol-O-methyltransferase (COMT)-deficient mice. *Eur J Neurosci* **15**, 246-256.

Johansen EB, Fonnum F, Lausund PL, Walaas SI, Baerland NE, Woien G, & Sagvolden T (2014). Behavioral changes following PCB 153 exposure in the spontaneously hypertensive rat - an animal model of Attention-Deficit/Hyperactivity Disorder. *Behav Brain Funct* **10**, 1.

Johansen EB, Knoff M, Fonnum F, Lausund PL, Walaas SI, Woien G, & Sagvolden T (2011). Postnatal exposure to PCB 153 and PCB 180, but not to PCB 52, produces changes in activity level and stimulus control in outbred male Wistar Kyoto rats. *Behav Brain Funct* **7**, 18.

Jones MW, Kilpatrick IC, & Phillipson OT (1986). The agranular insular cortex: a site of unusually high dopamine utilisation. *Neurosci Lett* **72**, 330-334.

Kennedy MB (1998). Signal transduction molecules at the glutamatergic postsynaptic membrane. *Brain Res Brain Res Rev* **26**, 243-257.

Kodavanti PR, Derr-Yellin EC, Mundy WR, Shafer TJ, Herr DW, Barone S, Choksi NY, MacPhail RC, & Tilson HA (1998). Repeated exposure of adult rats to Aroclor 1254 causes brain region-specific changes in intracellular Ca2+ buffering and protein kinase C activity in the absence of changes in tyrosine hydroxylase. *Toxicol Appl Pharmacol* **153**, 186-198.

Kodavanti PR & Tilson HA (1997). Structure-activity relationships of potentially neurotoxic PCB congeners in the rat. *Neurotoxicology* **18**, 425-441.

Lee MK, Tuttle JB, Rebhun LI, Cleveland DW, & Frankfurter A (1990). The expression and posttranslational modification of a neuron-specific beta-tubulin isotype during chick embryogenesis. *Cell Motil Cytoskeleton* **17**, 118-132.

Lisman JE & Grace AA (2005). The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron* **46**, 703-713.

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

Mariussen E & Fonnum F (2006). Neurochemical targets and behavioral effects of organohalogen compounds: an update. *Crit Rev Toxicol* **36**, 253-289.

Mergy MA, Gowrishankar R, Gresch PJ, Gantz SC, Williams J, Davis GL, Wheeler CA, Stanwood GD, Hahn MK, & Blakely RD (2014). The rare DAT coding variant Val559 perturbs DA neuron function, changes behavior, and alters in vivo responses to psychostimulants. *Proc Natl Acad Sci U S A* **111**, E4779-E4788.

Miller JJ, Richardson TL, Fibiger HC, & McLennan H (1975). Anatomical and electrophysiological identification of a projection from the mesencephalic raphe to the caudate-putamen in the rat. *Brain Res* **97**, 133-136.

Richardson JR & Miller GW (2004). Acute exposure to aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. *Toxicol Lett* **148**, 29-40.

Roth-Harer A, Lilienthal H, Bubser M, Kronthaler U, Mundy R, Ward R, Schmidt W, Winterhoff H, & Winneke G (2001). Neurotransmitter concentrations and binding at dopamine receptors in rats after maternal exposure to 3,4,3',4'-tetrachlorobiphenyl: the role of reduced thyroid hormone concentrations. *Environ Toxicol Pharmacol* **9**, 103-115.

Safe SH (1994). Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol* **24**, 87-149.

Sagvolden T & Johansen EB (2012). Rat models of ADHD. *Curr Top Behav Neurosci* **9**, 301-315.

Sazonova NA, DasBanerjee T, Middleton FA, Gowtham S, Schuckers S, & Faraone SV (2011). Transcriptome-wide gene expression in a rat model of attention deficit hyperactivity disorder symptoms: rats developmentally exposed to polychlorinated biphenyls. *Am J Med Genet B Neuropsychiatr Genet* **156B**, 898-912.

Schantz SL, Widholm JJ, & Rice DC (2003). Effects of PCB exposure on neuropsychological function in children. *Environ Health Perspect* **111**, 357-576.

Seegal RF, Brosch KO, & Bush B (1986a). Polychlorinated biphenyls produce regional alterations of dopamine metabolism in rat brain. *Toxicol Lett* **30**, 197-202.

Seegal RF, Brosch KO, & Bush B (1986b). Regional alterations in serotonin metabolism induced by oral exposure of rats to polychlorinated biphenyls. *Neurotoxicology* **7**, 155-165.

Seegal RF, Fitzgerald EF, Hills EA, Wolff MS, Haase RF, Todd AC, Parsons P, Molho ES, Higgins DS, Factor SA, Marek KL, Seibyl JP, Jennings DL, & McCaffrey RJ (2011). Estimating the half-lives of PCB congeners in former capacitor workers measured over a 28-year interval. *J Expo Sci Environ Epidemiol* **21**, 234-246.

Seegal RF, Marek KL, Seibyl JP, Jennings DL, Molho ES, Higgins DS, Factor SA, Fitzgerald EF, Hills EA, Korrick SA, Wolff MS, Haase RF, Todd AC, Parsons P, & McCaffrey RJ (2010). Occupational exposure to PCBs reduces striatal dopamine transporter densities only in women: a beta-CIT imaging study. *Neurobiol Dis* **38**, 219-225.

Seegal RF, Okoniewski RJ, Brosch KO, & Bemis JC (2002). Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: an in vivo microdialysis study. *Environ Health Perspect* **110**, 1113-1117.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, & Klenk DC (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**, 76-85.

Some M & Helander A (2002). Urinary excretion patterns of 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophol in various animal species: implications for studies on serotonin metabolism and turnover rate. *Life Sci* **71**, 2341-2349.

Surmeier DJ, Carrillo-Reid L, & Bargas J (2011). Dopaminergic modulation of striatal neurons, circuits, and assemblies. *Neuroscience* **198**, 3-18.

Tepper JM, Tecuapetla F, Koos T, & Ibanez-Sandoval O (2010). Heterogeneity and diversity of striatal GABAergic interneurons. *Front Neuroanat* **4**, 150.

Wigestrand MB, Stenberg M, Walaas SI, Fonnum F, & Andersson PL (2013). Non-dioxin-like PCBs inhibit [(3)H]WIN-35,428 binding to the dopamine transporter: a structure-activity relationship study. *Neurotoxicology* **39**, 18-24.

Williams JH & Ross L (2007). Consequences of prenatal toxin exposure for mental health in children and adolescents: a systematic review. *Eur Child Adolesc Psychiatry* **16**, 243-253.

Zahniser NR & Sorkin A (2009). Trafficking of dopamine transporters in psychostimulant actions. *Semin Cell Dev Biol* **20**, 411-417.

Zhang J, Vinuela A, Neely MH, Hallett PJ, Grant SG, Miller GM, Isacson O, Caron MG, & Yao WD (2007). Inhibition of the dopamine D1 receptor signaling by PSD-95. *J Biol Chem* **282**, 15778-15789.

Figures and Tables with legend

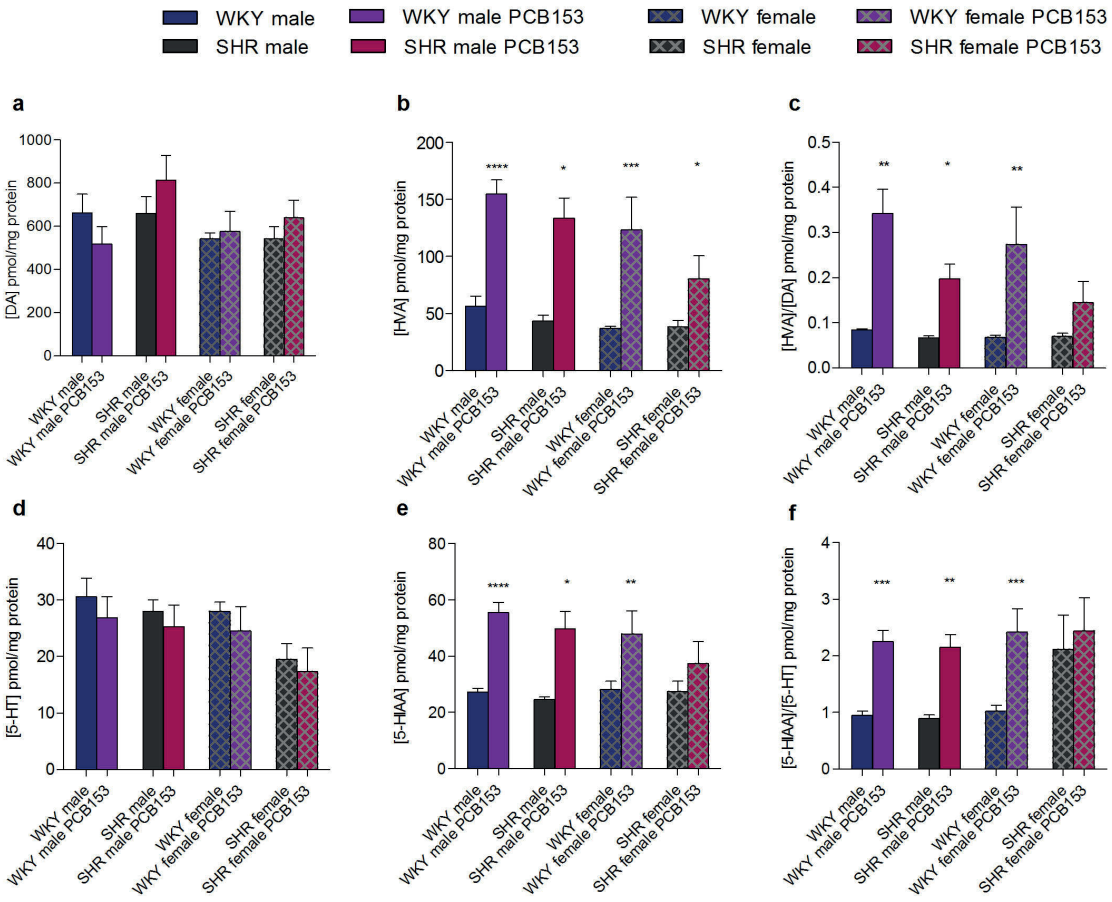

Figure 1: *PCB induced effects on neostriatal dopamine (DA) and serotonin (5-HT) levels and their metabolites in WKY and SHR rats.* Values are given in mean pmol/mg protein ± SEM. Values of **(A)** DA and **(B)** Homovanillic acid (HVA) was used to calculate **(C)** the DA turnover (HVA/DA). Values of **(D)** 5-HT and **(E)** 5-hydroxyindole acetic acid (5-HIAA) was used to calculate **(F)** the 5-HT turnover ratio (5-HIAA/5-HT)**.** n= 7-10 and *p < 0.05, **p < 0.01, when comparing exposed rats to their respective control.

	Amino acids nmol/mg protein								P - value			
									P-values: Control x PCB exposed			
	Male				Female				Male		Female	
	WKY (ctr)	WKY (PCB)	SHR (ctr)	SHR (PCB)	WKY (ctr)	WKY (PCB)	SHR (ctr)	SHR (PCB)	WKY	SHR	WKY	SHR
Glutamat	108 ± 6	109 ± 5	113 ± 10	112 ± 5	118 ± 8	121 ± 9	128 ± 8	114 ± 6	n.s	n.s	n.s	n.s
Glutamin	74 ± 5	71 ± 3	63 ± 4	69 ± 3	86 ± 4	88 ± 7	75 ± 6	73 ± 3	n.s	n.s	n.s	n.s
Aspartat	28 ± 4	36 ± 1	26 ± 3	34 ± 3	23 ± 1	39 ± 5*	31 ± 3	30 ± 1	n.s	n.s	0.03	n.s
Glycine	29 ± 2	29 ± 1	21 ± 1	26 ± 2	28 ± 2	24 ± 3	26 ± 1	24 ± 1	n.s	n.s	n.s	n.s
Serine	13 ± 0.6	15 ± 1.1	11 ± 0.6	15 ± 0.7	12 ± 0.9	15 ± 1	13 ± 1.3	14 ± 0.9	n.s	n.s	n.s	n.s
GABA	28 ± 1.6	25 ± 1.6	17 ± 1.6	25 ± 2.6	24 ± 1.9	29 ± 2.3	20 ± 3.0	23 ± 1.9	n.s	n.s	n.s	n.s
Taurine	126 ± 11	110 ± 5	96 ± 9	110 ± 4	99 ± 8	112 ± 7	110 ± 7	104 ± 4	n.s	n.s	n.s	n.s

Table 1: *PCB 153 does not induce changes in the levels of amino acids.* Levels of different amino acids in neostriatal preparations from control and PCB 153-treated rats are given as nmol/mg protein, from both genders of Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Data are presented as mean ± SEM. N = 7-10 and * $p < 0.05$.

Neostriatal homogenate															P - value			
	% of Male WKY ctr								Male				Female		P-values: Control x PCB exposed			
	Male				Female				WKY		SHR		WKY		SHR		Male		Female	
	WKY (ctr)	WKY (PCB)	SHR (ctr)	SHR (PCB)	WKY (ctr)	WKY (PCB)	SHR (ctr)	SHR (PCB)	Control	PCB	Control	PCB	Control	PCB	Control	PCB	WKY	SHR	WKY	SHR
TH	101 ± 16	90 ± 20	100 ± 14	101 ± 7	116 ± 10	96 ± 15	92 ± 18	119 ± 12									n.s	n.s	n.s	n.s
DAT	100 ± 3	72 ± 2*	102 ± 9	74 ± 12	133 ± 10	112 ± 15	148 ± 4	132 ± 8									<0.01	n.s	n.s	n.s
VMAT2	100 ± 13	116 ± 12	100 ± 19	129 ± 15	116 ± 34	150 ± 12	118 ± 17	166 ± 11									n.s	n.s	n.s	n.s
D5R	100 ± 7	60 ± 8*	100 ± 12	56 ± 14*	163 ± 3	115 ± 16*	97 ± 6	67 ± 5*									<0.01	<0.05	<0.05	<0.01
D1R	100 ± 2	97 ± 17	100 ± 14	95 ± 20	75 ± 8	91 ± 23	88 ± 12	80 ± 6									n.s	n.s	n.s	n.s
PSD-95	100 ± 5	59 ± 4*	101 ± 8	69 ± 11*	120 ± 16	112 ± 9	109 ± 9	113 ± 6									<0.01	<0.05	n.s	n.s
Na/K-ATPase	100 ± 8	87 ± 7	100 ± 20	76 ± 6	93 ± 8	98 ± 4	91 ± 10	98 ± 10									n.s	n.s	n.s	n.s
TUJ-1	100 ± 12	105 ± 10	100 ± 4	110 ± 4	83 ± 9	100 ± 9	103 ± 5	112 ± 5									n.s	n.s	n.s	n.s

Table 2: *PCB 153 induced changes in neostriatal proteins involved in dopaminergic transmission.* Western blot quantification of DA-associated neostriatal proteins and general protein markers are presented as % of levels expressed in the controls, respectively. The values of the proteins of female WKY and SHR controls are standardized against WKY male control rats. The values of tyrosine hydroxylase (TH), dopamine transporter (DAT) vesicular monoamine transporter-2 (VMAT-2) dopamine receptor D5/D1, postsynaptic density protein-95 (PSD-95), Na^+/K^+-ATPase and TUJ1 are listed in the eight columns to the left, as mean ± SEM. Examples of western blots are showed next to the P-values. n = 4 and *p < 0.05, **p < 0.01, when comparing exposed rats to their respective control.