Polychlorinated Biphenyls Alter Extraneuronal but Not Tissue Dopamine Concentrations in Adult Rat Striatum: An *in Vivo* Microdialysis Study

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Polychlorinated biphenyls (PCBs) reduce tissue dopamine (DA) concentrations and increase media DA concentrations in both *in vitro* preparations of bovine adrenal chromaffin cells and adult rat striatal tissue. To determine whether these changes also occur in the intact animal, we used *in vivo* microdialysis to determine changes in concentrations of DA in striatal dialysates from freely moving adult male rats after exposure to 25 mg/kg/day Aroclor 1254 for varying periods of time. We also determined DA concentrations in striatal tissue obtained postmortem from similarly treated animals. The effects of PCBs on dialysate DA concentrations depended on the length of exposure; DA concentrations were significantly elevated after 3 days of exposure and were significantly reduced after exposure for periods of 1 week or longer. On the other hand, striatal tissue concentrations of DA, determined postmortem in rats exposed to PCBs for the same periods of time, were not significantly altered. We suggest that these time-dependent alterations in dialysate DA concentrations *a*) reflect PCB-induced alterations of both plasma membrane and vesicular DA transporter function; *b*) provide a more sensitive index of altered central DA function after exposure to PCBs than does measurement of postmortem tissue DA concentrations; and *c*) play an important role in mediating some PCB-mediated changes in behavior. *Key words:* brain, dopamine, *in vivo*, microdialysis, polychlorinated biphenyls, striatum. *Environ Health Perspect* 110:1113–1117 (2002). [Online 18 September 2002]

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Polychlorinated biphenyls (PCBs) are widespread, persistent environmental toxicants that have been associated, particularly during development, with behavioral deficits in both humans and experimental animals (Jacobson and Jacobson 1996; Patandin et al. 1999; Roegge et al. 2000; Stewart et al. 2000) and with changes in biochemical and neurochemical function (Eriksson 1998; Goldey et al. 1995; Seegal et al. 1997).

The effects of PCB exposure on neurochemical function have been most extensively studied for the neurotransmitter dopamine (DA). Using pheochromocytoma (PC12) cells, we demonstrated that only noncoplanar PCB congeners significantly decreased cell DA content (Shain et al. 1991). Similar decreases in tissue DA content were described by Chishti et al. (1996) and by Bemis and Seegal (1999) after *ex vivo* exposure of striatal slices from adult rats to commercial mixtures of PCBs. These decreases in tissue DA content after acute exposure were accompanied by significant elevations in media DA, which led us to suggest that PCBs either enhance release of DA and/or inhibit transport of the neurotransmitter back into the neuron. Similar results have also been seen by other investigators; Messeri et al. (1997) reported increased release of catecholamines into medium after exposure of bovine adrenal chromaffin cells to the noncoplanar PCB congener 2,4,2′,4′-tetrachlorobiphenyl (TCB) but not to the tetrachlorinated coplanar congener 3,4,3′,4′-TCB.

These elevations in media DA concentrations may involve PCB-mediated inhibition of the membrane dopamine transporter (DAT). Indeed, Rosin and Martin (1981) reported significant reductions in uptake of biogenic amine neurotransmitters into synaptosomes after exposure to Aroclor mixtures, whereas Mariussen and Fonnum (2001) recently reported that only noncoplanar PCB congeners inhibited uptake of labeled DA into striatal synaptosomes. Taken together, these findings from disparate neuronal preparations suggest that noncoplanar PCB congeners affect the release and/or uptake of neurotransmitters, DA in particular, that play important roles in mediating behavior (Arnsten 1997; Sokolowski and Salamone 1994).

However, the above-mentioned neurochemical studies are *in vitro* studies and, to the best of our knowledge, no studies have determined whether these findings can be extrapolated to the more complex *in vivo* neuronal environment. We therefore exposed adult male rats to a commercial mixture of PCBs (Aroclor 1254) and used *in vivo* microdialysis to determine extraneuronal (i.e., dialysate) concentrations of DA. Furthermore, to determine whether these changes in dialysate DA concentrations provide a more sensitive index of altered DA function than do changes in postmortem tissue DA concentrations, we measured striatal tissue DA concentrations in male rats of the same age, exposed to PCBs for identical periods of time.

Materials and Methods

Animals. Experimental subjects consisted of adult male Sprague-Dawley–derived rats either from the breeding facilities of the New York State Department of Health or from Taconic Farms (Germantown, NY). No statistical differences in body weight or neurochemical responses to PCBs were detected between the Sprague-Dawley rats from the two different sources.

Animals were approximately 12 weeks of age before their initial exposure to PCBs and were housed singly in plastic cages (45 × 24 × 16.5 cm) with Sani-Chips heat-treated bedding (P.J. Murphy Forest Products, Montville, NJ) in a secure facility maintained at 23°C on a 12-hr light:12-hr dark schedule. There were three or four PCB-exposed and control animals at each exposure period for *in vivo* microdialysis, and three to six PCB-exposed and control animals at each exposure period for determination of postmortem striatal tissue DA concentrations.

PCBs. Aroclor 1254 was obtained from the Wadsworth Center, New York State Department of Health, and was analyzed by high-resolution mass spectrometry (O'Keefe et al. 1985) for polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). Total PCDFs were 22.4 ppm, and no PCDDs were found at detection limits of 0.3 ppb.

Exposure of animals to PCBs. We provided each animal with one-half of a vanilla wafer cookie containing a sufficient amount of Aroclor 1254 dissolved in corn oil to result in a daily PCB exposure of 25 mg/kg/day. Animals were exposed on a daily basis to either Aroclor 1254 (PCB-exposed) or corn oil (control) for 3 days or 1, 2, or 8 weeks. We have used this procedure in the past (Seegal et al. 1997) and have found that it is a nonstressful means of providing a known quantity of PCBs to the animal. This procedure was used for all animals, including those that underwent microdialysis and those sacrificed for determination of

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postmortem striatal tissue DA concentrations. PCB exposure was continued until the day microdialysis was conducted for all animals. Thus, except for those exposed to either PCBs or corn oil for 3 days, exposure to either PCBs or corn oil began before surgical implantation of the guide cannula. An experimental time line of the relationship between PCB exposure, surgical implantation of a guide cannula, and microdialysis is presented in Figure 1.

Surgical procedures for microdialysis. All surgical and postoperative procedures were approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. Adult male rats were anesthetized (65 mg/kg sodium pentobarbital, intraperitoneally) and placed in a Kopf model 900 stereotaxic instrument (David Kopf Instruments, Tujunga, CA). Using the stereotaxic atlas of Pellegrino et al. (1981), we lowered a guide cannula into the striatum using the following coordinates: anterior–posterior, +2.4 mm; lateral, +3.0 mm (both with respect to bregma); and dorsal–ventral, –3.0 mm, with respect to the brain surface. The guide cannula was attached to the skull using dental acrylic anchored by several small stainless steel screws embedded in the skull. Postoperative pain was minimized by administration of two intramuscular injections of 0.2 mg/kg Torbugesic-SA (butorphanol tartrate; Fort Dodge Animal Health, Fort Dodge, IA) at 4–6-hr intervals. All animals were observed on a daily basis and were allowed a 5–7-day recovery period before insertion of the microdialysis probe.

Microdialysis procedures. On the afternoon before microdialysis, animals were anesthetized intraperitoneally using 35–50 mg/kg of Brevital (methohexital sodium; Jones Pharma, Inc., St. Louis, MO), a short-acting anesthetic agent. A CMA12 microdialysis probe (CMA/Microdialysis, North Chelmsford, MA) with a 3-mm dialysis-probe length was inserted into the guide cannula, and the animal was placed in a circular cage fitted with a commercially available counterweighted liquid swivel (Instech Laboratories, Inc., Plymouth Meeting, PA). Artificial cerebrospinal fluid (148 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2 \cdot 2H_2O$) was pumped through the probe at a rate of 0.5 µL/min overnight, and food and water were provided *ad libitum*. The next morning, the flow rate was increased to 1 µL/min, and 30-min dialysate samples were collected for neurochemical analyses. Each dialysate sample was collected in a sample tube containing 3 µL of a preservative consisting of 2.2 N perchloric acid containing 150 µM Na_2EDTA and 290 µM $Na_2S_2O_5$ and was immediately stored at 4°C until analysis later that day.

Determination of striatal tissue DA concentrations. After sacrifice, using institutionally approved procedures, brains were rapidly removed, rinsed with ice-cold saline, blotted, quickly frozen on powdered dry ice, wrapped tightly in aluminum foil, and maintained at –80°C until dissection and subsequent neurochemical analysis. For the micropunch brain dissection technique, the brains were brought to –10°C and mounted using TissueTek (Sakura Finetek U.S.A., Inc., Torrance, CA) on an aluminum chuck maintained on dry ice. Brain tissue was cut into 750-µm-thick sections, mounted on glass slides, and placed on powdered dry ice. Tissue punches 1.6 mm in diameter were obtained from the striatum using the atlas of Palkovits and Brownstein (1988). Tissue punches were homogenized in 150 µL of 0.2 N perchloric acid containing 100 mg/L EGTA using an ultrasonic homogenizer and centrifuged in a microcentrifuge for 60 sec at 2–4°C. Homogenates were frozen at –80°C until the time of analysis (within 2 weeks).

HPLC analysis of DA. Concentrations of DA, in either microdialysates or perchloric acid supernatants of striatal tissue homogenates, were determined using chromatographic separation methods developed and/or modified in our laboratory (Bemis and Seegal 1999; Nakamura et al. 1992; Seegal et al. 1986a). Aliquots (20 µL) of striatal microdialysates or of a 1:10 dilution of striatal tissue homogenates were injected via a Waters 717 refrigerated autosampler (Waters Corp., Milford, MA) onto a SUPELCOSIL LC-18-DB 25 cm × 4.6 mm, 5-µm C18 reverse-phase column (Supelco, Bellefonte, PA) maintained at 35°C. DA was quantified using a BAS LC-4C amperometric detector (BioAnalytical Systems, Inc., West Lafayette, IN). Each chromatogram was stored digitally, individually reviewed, and analyzed using Waters Millennium Chromatography Manager Software (Waters Corp.). Data from the animals that underwent microdialysis were expressed as nanograms of DA per collection period and were corrected for probe efficiency (Kehr 1993), and the postmortem tissue DA content was expressed as nanograms per milligram of protein, determined by the method of Lowry et al. (1951).

Statistical procedures. Two-way analysis of variance (ANOVA) with post hoc *t*-tests was used to determine the significance of differences in the body weights of control animals and animals exposed to PCBs for 3 days or for 1, 2, or 8 weeks.

For the microdialysis experiments, the data from control animals at the different exposure times were combined because there were no statistical differences between control animals at any of the exposure periods. The effects of exposure to Aroclor 1254 on dialysate DA concentrations were initially analyzed using a three-way ANOVA, which examined the effects of PCBs and duration of exposure (both between-subject variables), as well as the within-subject repeated measure of the four collection periods. Because the three-way analysis does not permit determination of which durations of exposure to PCBs differ from controls, we also carried out separate two-way ANOVAs (with repeated measures for the four 30-min collections) for each exposure duration (i.e., 3 days or 1, 2, or 8 weeks). One-way ANOVAs were used to determine the effects of PCB exposure on postmortem striatal tissue concentrations of DA.

Results

Effects of PCB exposure on body weight. The effects of daily exposure to 25 mg/kg Aroclor 1254 on body weights of adult male rats are shown in Figure 2. Only after 8 weeks of exposure to PCBs were there significant differences in body weight between PCB-exposed and control animals [$t = 5.97$, degrees of freedom (df) = 17, $p \leq 0.001$].

Effects of PCB exposure on dialysate DA concentrations. The three-way ANOVA failed to show a significant main effect of PCBs (because of the initial increase in dialysate DA seen at 3 days, followed by later reductions). However, the two-way interaction term of PCB × exposure duration was significant ($F = 5.46$, $df = 3,18$, $p < 0.01$), demonstrating that

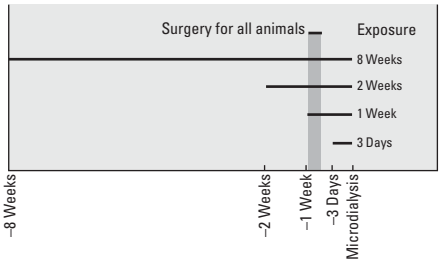

Figure 1. Experimental time line of the relationship between PCB exposure, surgical implantation of a guide cannula, and microdialysis.

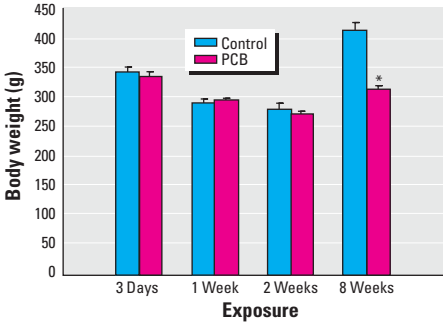

Figure 2. Body weights of animals used for microdialysis and striatal punch experiments. Control animals and animals exposed to 25 mg/kg/day Aroclor 1254 were sacrificed for experimental time points at 3 days or at 1, 2, or 8 weeks. n = 7–11 animals/condition. Error bars indicate SE.
*$p \leq 0.001$, post hoc *t*-test comparing PCB-exposed and control animals.

dialysate DA concentrations depended on the duration of exposure to PCBs.

Subsequent two-way ANOVAs demonstrated that the effects of exposure to Aroclor 1254 on dialysate DA concentrations supported that finding. Exposure for 3 days significantly increased ($F = 13.12$, $df = 1,15$, $p \leq 0.01$) dialysate DA concentrations compared with control animals (Figure 3), whereas exposure for longer periods of time significantly decreased dialysate DA concentrations (1 week: $F = 5.75$, $df = 1,14$, $p \leq 0.05$; 2 weeks: $F = 9.85$, $df = 1,14$, $p \leq 0.01$; 8 weeks: $F = 5.35$, $df = 1,14$, $p \leq 0.05$; Figure 3).

Effects of PCB exposure on striatal tissue DA concentrations. Striatal tissue DA concentrations, determined postmortem in rats exposed to PCBs for the same periods of time as animals that underwent *in vivo* microdialysis, were not significantly altered after exposure to Aroclor 1254 at any of the time points examined (Figure 4).

Discussion

Our major finding in this study was that exposure of the adult rat to fairly low concentrations of PCBs significantly altered dialysate (extraneuronal) DA concentrations—increasing DA concentrations after 3 days and decreasing DA concentrations after longer periods of exposure—without altering tissue DA concentrations. These results demonstrate that *a*) the mature central nervous system may be more sensitive to PCBs than previously thought; *b*) *in vivo* microdialysis provides a more sensitive measure of PCB-induced change in DA function than does measurement of tissue DA concentrations; and *c*) changes in dialysate DA concentrations depend on the duration of exposure to PCBs.

The elevations in dialysate DA concentrations seen here are strikingly similar to those seen in media DA concentrations after acute *ex vivo* exposure of adult rat striatal tissue to Aroclor mixtures (Bemis and Seegal 1999; Chishti et al. 1996) and are likely to reflect similar mechanisms. Indeed, in those reports, we suggested that the elevations in media DA may have been due to PCB-induced inhibition of DA transporters. The short-term elevation in dialysate DA concentrations most likely involves PCB-induced inhibition of the DAT because drug-induced DAT inhibition significantly elevates dialysate DA concentrations in rat striatum (Nakachi et al. 1995), whereas pharmacologic inhibition of the vesicular monoamine transporter (VMAT) has little or no consequences on DA overflow in striatal slices (Jones et al. 1998). However, it is perhaps premature to rule out other mechanisms that may occur *in vivo* and that may involve either inhibition of DA uptake into intraneuronal vesicles (Mariussen et al. 1999) (leading to elevations in cytosolic free DA) or PCB-induced elevations in intracellular calcium concentrations (Kodavanti et al. 1996). In turn, elevations in intracellular calcium enhance exocytotic release of vesicular DA (Bonanno et al. 2000) into the extraneuronal space, further elevating extraneuronal DA due initially to DAT inhibition.

We suggest that the long-term decreases in striatal dialysate DA concentrations may be a consequence of PCB-induced elevations in both dialysate and cytosolic DA concentrations for two reasons. First, elevations in extraneuronal DA (seen here at 3 days and most likely due to DAT inhibition) activate synthesis regulating autoreceptors, partially inhibiting *de novo* DA synthesis (Wolf and Roth 1990) and thus reducing the pool of DA available for release. Second, elevations in free cytosolic DA, measured as increases in dialysate DOPAC (3,4-dihydroxyphenylacetic acid) concentrations, (Teng et al. 1997; Zetterstrom et al. 1988), and most likely due to VMAT inhibition, may further contribute to reductions in *de novo* synthesis of DA by altering the affinity of tyrosine hydroxylase (TH) for its cofactor, tetrahydrobiopterin (Cooper et al. 1996; Minami et al. 1992).

The rapid changes in dialysate DA concentrations (which may have occurred even earlier than we measured) suggest that, at least for the adult rodent, this widely dispersed environmental neurotoxicant may have "pharmacologic" properties (i.e., PCBs may induce changes in neurochemical function on a time scale similar to that of some neuroactive drugs). Indeed, the biphasic changes in dialysate DA concentrations after

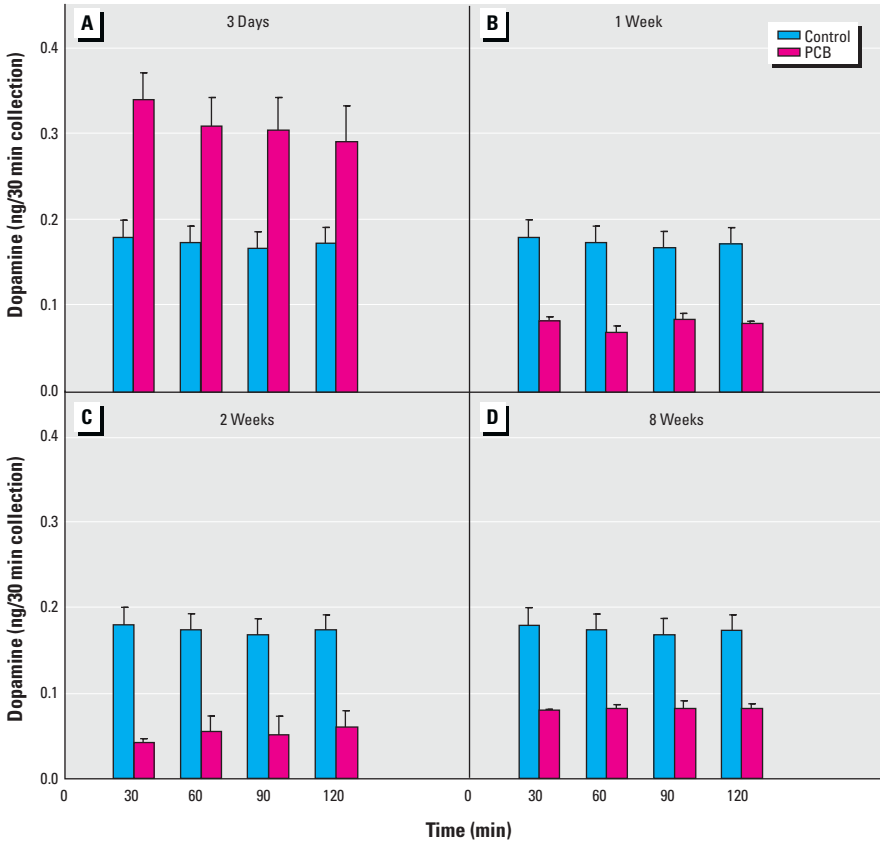

Figure 3. Effects of Aroclor 1254 on extracellular DA concentrations in controls or in animals exposed to 25 mg/kg/day Aroclor 1254 (PCB) for (*A*) 3 days ($p \leq 0.01$), or for (*B*) 1 week ($p \leq 0.05$), (*C*) 2 weeks ($p \leq 0.01$), or (*D*) 8 weeks ($p \leq 0.05$) prior to microdialysis. Four microdialysates (30 min/sample) were collected from each animal; $n = 3$–13 animals/condition. Data are expressed as nanograms of DA per 30-min collection interval, corrected for microdialysis probe efficiency. Data were analyzed at each exposure interval using two-way ANOVA with repeated measures for the collections. Error bars indicate SE.

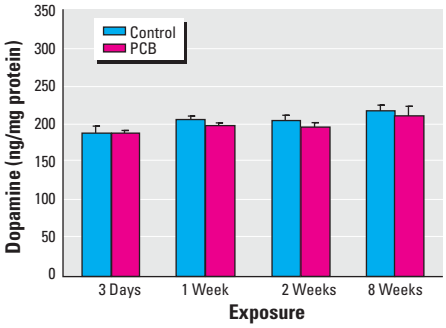

Figure 4. DA concentrations in striatal brain micropunches from controls or from animals exposed to 25 mg/kg/day Aroclor 1254 for 3 days or for 1, 2, or 8 weeks. $n = 4$–7 animals/condition. Error bars indicate SE.

exposure to PCBs—in terms of both timing and magnitude—are strikingly similar to the changes seen by Imperato et al. (1996) after subchronic exposure to *d*-amphetamine (*d*-AMPH). The short-term elevations in *d*-AMPH–induced dialysate DA concentrations are likely to be mediated by mechanisms similar to those by which PCBs elevate media or extraneuronal concentrations of DA (i.e., VMAT and DAT inhibition). Thus, Jones et al. (1998) demonstrated that *ex vivo* exposure of striatal slices to *d*-AMPH resulted in VMAT inhibition, reversal of normal DAT function (i.e., transport of cytosolic DA into the extracellular space), and enhanced release of DA into media. However, at present there is no evidence suggesting that PCBs reverse DAT function.

The rapid changes in dialysate DA concentrations after exposure of the intact adult rat to relatively modest levels of PCBs stand in sharp contrast to the longer periods of time and higher concentrations of PCBs required to induce significant changes in tissue DA concentrations (Seegal et al. 1986b, 1991). Although seemingly counterintuitive, there are several likely explanations for the lack of effect of PCBs on tissue DA concentrations seen here. First, the dose of PCBs used in this study is considerably smaller than that used in other studies that have demonstrated reductions in brain DA concentrations in adult rats exposed to PCBs (Seegal et al. 1991). Second, the lack of discernible effect of PCBs on tissue DA concentrations may in part be because of the existence of two pools of DA—a readily releasable pool (RRP) of DA replenished by *de novo* synthesis and a larger, non-readily releasable storage pool (Chiueh and Moore 1975; Kuczenski 1977; Yavich and MacDonald 2000) [estimates of the size of the RRP of DA vary between 5% and 20% of total DA (Javoy and Glowinski 1971; Yavich 1996)]. Because tissue DA includes contributions from both pools while extraneuronal DA reflects changes in only the RRP of DA, the consequences of PCB exposure would be more apparent in the smaller pool. Furthermore, PCB-induced inhibition of DA synthesis (due either to activation of inhibitory autoreceptors or end-product inhibition due to elevations in cytosolic DA) would have consequences primarily on the RRP because the vesicular content of the RRP of DA under unstimulated conditions is replenished with DA that is either newly synthesized or recently taken up from the extraneuronal space by the DAT (Javoy and Glowinski 1971; McMillen et al. 1980). Finally, PCB-induced VMAT inhibition is more likely to reduce DA concentrations in the RRP than in the larger storage pool because of the higher turnover of DA (i.e., release and reuptake) in the RRP (McMillen et al. 1980).

There are several possible consequences of the PCB-induced inhibition of DA transporters. First, increased cytosolic concentrations of free DA (i.e., that not stored in vesicles and due to VMAT inhibition) enhance both enzymatic and nonenzymatic degradation of DA, leading to an increase in the formation of reactive DA metabolites (LaVoie and Hastings 1999), including quinones and semiquinones shown to inhibit DAT function (Berman et al. 1996). These quinones, as well as other free radicals (Voie and Fonnum 2000), including hydrogen peroxide (Miller et al. 1996), may ultimately lead to an increased likelihood of free radical-induced neuronal damage. However, at the doses and durations of exposure employed in this study, it is unlikely that the PCBs lead to neuronal death because *a*) tissue DA concentrations were not altered and *b*) lactate dehydrogenase levels were unaffected in organotypic striatal slice preparations from adult rats exposed to higher concentrations of PCBs than used here (Bemis and Seegal 1999). Second, a reduction in the vesicular concentration of DA [similar to that seen by Jones et al. (1998) and Imperato et al. (1996) after long-term exposure to *d*-AMPH] may result in a decrease in the amount of DA released after depolarization of the neuron, thereby altering the normal transfer of information between neurons.

Although these studies were undertaken in the adult animal, they may ultimately aid in furthering our understanding of some of the mechanisms by which developmental exposure to PCBs alters neuronal function. Before this study, the effects of PCBs on *in vivo* DA function were estimated by measuring either tissue DA concentrations (Seegal et al. 1986b, 1991) or TH (Kodavanti et al. 1998; Seegal et al. 1994). Indeed, Kodavanti et al. (1998) exposed adult rats to concentrations of PCBs similar to those used here but were unable to detect differences in either tissue DA concentrations or TH immunoreactivity. These findings raise the toxicologically important question of whether the disparity between the concentrations of PCBs needed to alter neurochemical function and those required to alter some behaviors may reflect the earlier methods used to assess central DA function, which we have shown to be relatively insensitive.

In conclusion, the results that we obtained using *in vivo* microdialysis demonstrate that changes in adult striatal dialysate DA occur at much earlier times and at significantly lower doses of PCBs than do changes in adult striatal tissue DA content determined postmortem. Furthermore, we suggest that the PCB-induced changes in dialysate DA concentrations are strikingly similar to those seen after exposure to *d*-AMPH. Thus, cognitive deficits, including decreased attention span, that are purported to be due to developmental exposure to PCBs may, in part, be due to alterations in DA function similar to those that induce deficits in infants and children developmentally exposed to stimulant drugs of abuse.

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