Exposure to High Doses of the di-*ortho*-Substituted Polychlorinated Biphenyls 153 and 180, But Not 52, Leads to Behavioural Changes in Rats

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This present study is part of a bigger study in professor Sagvolden’s research group at the Department of Physiology at the University of Oslo.

Summary

Background: Polychlorinated Biphenyls (PCBs) are a group of toxins that are hydrophobic and lipophilic. Acute and chronical exposure through mother's milk and food consumption can produce hyperactivity and disturbances in cognitive development in humans. Animal studies have shown inconsistent changes in behaviour and attention. More consistent findings of reductions in dopamine levels have also been found. This makes PCB exposure relevant for certain dopamine based diseases, such as Parkinson's disease (PD) and Attention Deficit/Hyperactive Disorder (ADHD).

Methods: Male outbreed Wistar Kyoto (WKY/NHsd) rats were exposed to 10 mg/kg PCBs 52, 153 or 180 dissolved in corn oil by gavage three times between PND 10 and 20. A control group were given pure corn oil. At postnatal day (PND) 35 the rats started testing on a RI EXT reinforcement schedule measuring activity, impulsivity and sustained attention.

Results: Rats exposed to PCB 153 or 180 showed significantly less activity and impulsive behaviour than the controls and PCB 52 group. The PCB 153 and 180 groups also performed better on the sustained attention measure. No such results were found for the PCB 52 group.

Discussion: It is suggested that the high dose of exposure to the highly chlorinated congeners PCB 153 and PCB 180 produced motor problems in the rats causing hypoactivity. The effect thus more resembles PD symptoms than ADHD. Further research is recommended on dose-response curves and animal models of the disorders investigating a possible genetic vulnerability factor.

Introduction

Polychlorinated Biphenyls (PCBs) belong to a group of stable and heat-resistant halogenated aromatic hydrocarbons (HAHs) (Bushnell & Rice, 1999; Giesy & Kannan, 1998). The PCB molecule consists of two phenyl rings (biphenyl) each consisting of six carbon atoms where chlorine atoms may be substituted for hydrogen at any numbered position giving a total of 209 possible structures (congeners) (Smith & Gangolli, 2002). All the different congeners have variations of the same formula: $C_{12}H_{10-x}Cl_x$ (Fig 1a).The congeners are named according to the positions and numbers of chlorine atoms, e.g. 2,2',5,5'-tetrachlorobiphenyl, and/or by congener numbers as applied by the International Union of Pure and Applied chemistry (IUPAC), e.g. PCB 52 (Rice, 1997a). For PCB 52 the formula is $C_{12}H_6Cl_4$ (Fig 1b).
Figure 1 shows a schematic description the biphenyl molecule and of PCB 52.

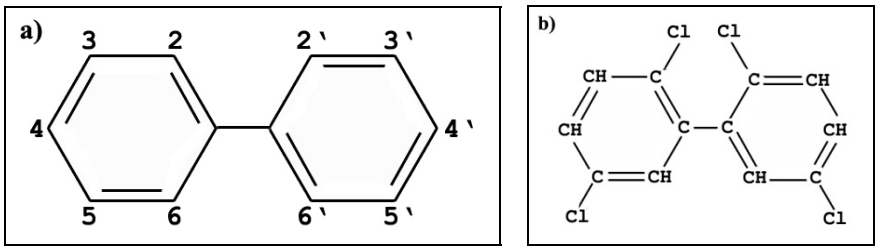

Figure 1 a) Biphenyl with the substitute positions marked, b) PCB 52 (2,2',5,5'-tetrachlorobiphenyl) molecule, $C_{12}H_6Cl_4$.

The different PCB congeners can be divided into three main structural classes: (1) coplanar, dioxin-like PCBs having chlorine substitution in the *para* (4, or 4') and *meta* (3, 3', 5 or 5') positions and no chlorine substitution in the *ortho* (2, 2', 6 or 6') position (Fig 1a); (2) *mono-ortho*-substituted PCBs having one chlorine substitution at the *ortho* position and may attain coplanarity (the whole molecule is in the same plane); and (3) noncoplanar PCBs having two or more *ortho* substitutions (Mariussen & Fonnum, 2006). PCB 52 having chlorine substitutions in both the 2 and 2' position is thus a di-*ortho*-substituted noncoplanar PCB congener.

PCBs are resistant to acids and bases, compatible with organic materials, resistant to oxidation and reduction, thermally stable and nonflammable (Schantz, 1996). Because of these characteristics they have been widely used as nonflammable alternatives to mineral oils for capacitors and transformers in the electrical industry (Bushnell et al., 2002), as dielectic

fluids, heat exchangers, hydraulic oils, paint additives and plasticizers (Bushnell et al., 1999), and as coolants and lubricants in electrical equipment (Branchi et al., 2005).

In commercial use, the congeners consist mainly in mixtures. These can be identified according to their chlorine content, e.g. Aroclor 1254 (A1254) has 54% chlorine by weight. In addition to the American Aroclors, other mixtures include Chlophens (Germany), Phenoclors and Pyralenes (France), Fenclors (Italy) and Kanechlors (Japan) (Giesy et al., 1998). PCBs found in the environment originate primarily from these commercially produced mixtures, but the persistence of the involved congeners varies, with the more highly chlorinated PCB congeners tending to be the least water soluble (Giesy et al., 1998) and also the most biologically stable (Roegge, Seo, Crofton, & Schantz, 2000).

PCBs were manufactured in the United States from 1929 until production was banned in 1977 (Bushnell et al., 2002) subsequently followed by bans in Western Europe. In Eastern Europe and Russia production continued until early 1990s (Giesy et al., 1998).

Despite the bans, PCBs are still present in the environment as a worldwide pollution problem, and they keep accumulating in the food chain (Ulbrich & Stahlmann, 2004; Rice, 1995). The characteristics that made the chemical ideal for the industrial world are, paradoxically, the same that make it resistant to degradation and able to bioaccumulate in the environment (Schantz, 1996). They will continue to pose a potential risk of exposure through accidents, improper disposal practices, transport of equipment containing PCBs, and through contaminated animals and food for human consumption (Chen, Yu, Rogan, Gladen, & Hsu, 1994).

PCBs are lipophilic and hydrophobic, i.e. fat but not water soluble, and thus become more concentrated as they move higher in the aquatic food chain, contaminating fish in exposed waters such as the Great Lakes and Hudson River (Stewart, Reihman, Lonky, Darvill, & Pagano, 2000). These fish are then part of the human food chain exposing those consuming them to greater than background levels of PCBs (Daly, Hertzler, & Sargent, 1989). Contaminated fish might, therefore, be seen as the most significant source of the chemical for the general population (Rice, 1995). It is estimated that over 90% of the exposure occurs through the diet (Smith et al., 2002).

Since PCBs are lipophilic and stored in fat tissue, the chemicals also occur at much higher levels in milk than in blood (Rice, 1997a). Mother’s milk provides an excretion pathway for the mother, but at the same time an exposure route for the child (Inoue et al., 2006). Foetuses and nursing infants thus receive significant exposure to PCBs and other organochlorines at higher levels than the adult population (Hooper & McDonald, 2000). In

addition, studies suggest that the human nervous system during development is particularly sensitive to exposure to PCBs and related chemicals (Kodavanti, 2005). Young children also spend a lot of time on the floor stirring up and breathing in dust and residues potentially further exposing them to toxins (Weiss, 2000).

The PCB congeners found most often in humans are the *ortho*-chlorinated PCBs 153, 118, 138, and 180 (Longnecker et al., 2003; Korrick et al., 2000). The more highly chlorinated congeners are less soluble and their means of prevalence therefore exceeds the means for the lower chlorinated congeners (Schwartz, Jacobson, Fein, Jacobson, & Price, 1983). The less highly chlorinated congeners have also been found, such as PCB 52, but at lower concentrations (Korrick et al., 2000).

Effects of human exposure

The potential dangers PCB exposure and contamination pose for humans were first discovered after two acute and accidental exposures of PCBs mixed with rice bran oil. The first incident was in Japan 1968, sometimes referred to as Yushu, where the rice oil was contaminated by PCBs during the manufacturing process (Schantz, 1996). 11 years later a similar accident occurred in Taiwain, the Yu-Cheng (Rogan et al., 1988).

Yamashita and Hayashi found that pregnant women exposed in Japan delivered babies with certain mutual clinical symptoms they called fetal PCB syndrome (FPS). The children had dark brown pigmentation (“coca-cola color”), gum enlargement (gingival hyperplasia), eyes bulging out of orbit (exophthalmic edematous eye), teeth at birth, abnormal calcification of the skull, a structural malfunction in the foot (rocker bottom heel) and higher incidents of low birth weight (Yamashita & Hayashi, 1985). Physical deficits largely resembling the fetal PCB syndrome in Japan were also found amongst the in utero exposed Yu-Cheng babies in Taiwan (Rogan et al., 1988).

The Yu-Cheng children also showed a delay in developmental milestones, deficits on formal developmental testing and abnormalities on behavioural assessment. (Rogan et al., 1988; Yu, Hsu, Gladen, & Rogan, 1991). Followed biannually from 1985-1991, the children showed more health problems, increased activity levels and slower cognitive development than controls. This was also found in children born up to six years after their mothers had been exposed (Chen et al., 1994).

The chemicals in the contaminated oils in both incidents were not limited to PCBs only. Both incidents also had exposure to high concentration of dibenzofurans which are

much more toxic than PCBs (Schantz, 1996) and to PCDF (Kodavanti, 2005). However, in an analyses of 36 available subjects, 20 children had detectable PCBs, while no PCDFs were found (Yu et al., 1991).

In addition to research on children born after the Yushu and Yu-Cheng, epidemiological studies have been conducted on more chronical exposure. In utero PCB exposure in newborns and toddlers has been found to be associated with neurological and cognitive functioning in North America and the Netherlands (Stewart et al., 2000; Patandin et al., 1999; Huisman et al., 1995). A cross-sectional study from the Maastricht Aging Study also found that exposure to pesticides was associated with increased risk of mild cognitive dysfunction (MCD) (Bosma, van Boxtel, Ponds, Houx, & Jolles, 2000).

In studies of children exposed to PCBs in utero and via mother's milk by Lake Michigan, relations between PCB exposure and increased impulsivity, reduced concentration, poorer working memory and lower IQ scores were found (Jacobson & Jacobson, 2003; Jacobson & Jacobson, 1996). The in utero PCB concentrations in this sample were only slightly higher than those in the general population indicating that even small increases in PCB exposure can have a long-term impact on intellectual function.

More impulsive behaviour and impaired response inhibition have also been found amongst children living by Lake Ontario and on the Faroe Islands (Stewart et al., 2005; Grandjean et al., 2001).

Together these and other studies show that PCBs exposure in humans is associated with motor and cognitive deficits, and potential risks for developmental disturbances (Kodavanti, 2005; Guvenius, Aronsson, Ekman-Ordeberg, Bergman, & Noren, 2003). They cannot, however, give definitive answers of cause and effect. An extensive amount of research has therefore been done on animals to shed light on the causality of PCB and developmental deficits.

Animal studies

The designs in the animal studies have varied a great deal making it hard to summarize and draw conclusions. Important variables influencing the effects of PCB exposure include the PCBs used (commercial mixtures, single congeners), route of exposure (in utero, through mother's milk, gavage, fish), type of testing (reinforcement schedules, mazes etc), age at exposure, age at testing and type and sex of animal used. The following findings are reviewed according to the origin of PCB exposure.

Rats fed PCB contaminated fish from the St. Lawrence River showed hyperactivity and bursts of responses with short inter response times (IRTs), i.e. short time intervals between responses (Berger et al., 2001). The average response rates in the exposed rats were more than 1.5 times that of the controls.

Studies using PCB mixes resembling the congeners found in mother's milk or contaminated fish have also been conducted. In a series of studies, monkeys were exposed to a PCB mixture based on milk between birth and 20 weeks of age. The subjects showed problems on complex learning tasks and struggled to recognize that responses were required to change in a systematic manner (Rice, 2000; Rice, 1999; Rice, 1997b). Adult rats became hyperactive and produced an increased number of short IRTs after in utero exposure to a PCB mixture resembling that in fish from Fox River in Green Bay (Sable, Powers, Wang, Widholm, & Schantz, 2006).

Exposure to one of the more highly chlorinated Aroclor mixes, A1254, during gestation and lactation caused hyperactivity in mice, mainly manifested in adulthood (Branchi et al., 2005). Adult exposure to A1254 was associated with a dose-dependent hypoactivity in mice (Nishida, Farmer, Kodavanti, Tilson, & MacPhail, 1997). The mixture also produced working and reference memory deficits in rats tested on a radial arm maze (Roegge et al., 2000). However, perinatal exposure to A1254 also failed to show any effect in young or adult rats either on habituation of motor activity, acquisition of visual discrimination or performance on a visual signal-detection task (Bushnell et al., 2002).

Exposure to the mixture A1248 showed increased activity and higher number of short IRTs in rats similar to that of the rats fed St. Lawrence River fish (Berger et al., 2001). Both A1248 and A1016 also produced a facilitatory effect on the shape problem in a two-choice discrimination-reversal task in monkeys exposed through gestation and lactation (Schantz, Levin, Bowman, Heironimus, & Laughlin, 1989).

Studies have also been conducted using single congeners as source of exposure. Rats exposed to the relatively highly chlorinated di-*ortho*-substituted PCB 153 or the non-*ortho*-substituted PCB 126 through mother's milk displayed higher activity levels and attentional deficits on a reinforcement task. The males exposed to PCB 153 also showed "bursts" of lever presses with short IRTs (Holene, Nafstad, Skaare, & Sagvolden, 1998). When tested on a Y-maze , young rats exposed to either one of the two same congeners during gestation and lactation showed impaired learning compared to a control group (Piedrafita, Erceg, Cauli, Monfort, & Felipo, 2008).

Exposure to the non-*ortho*-substituted congeners PCB 126 or PCB 77 produced a decrease in working memory errors in rats tested on a radial arm maze (Schantz, Seo, Moshtaghian, Peterson, & Moore, 1996), while exposure to the *ortho*-substituted PCBs 153 or 28 or the non-*ortho*-substituted PCB 118 produced slower acquisition in female rats on a T-maze spatial alternation task (Schantz, 1995). However, using the congener PCB 126, Bushnell and Rice did not find any attentional changes in rats (Bushnell et al., 1999).

Hyperactivity has been found in mice given the PCB 77 (Agrawal, Tilson, & Bondy, 1981). Mice exposed to the *ortho*-substituted PCB 52 or a combination of lower doses of PCB 52 and the Polybrominated Diphenyl Ether (PBDE) 99 showed impaired spontaneous motor behaviour and habituation capacity, indicating an interaction effect between PCBs and other toxins (Eriksson, Fischer, & Fredriksson, 2006).

The use of animals not only makes it possible to investigate causality on behavioural measures, but also allow for experiments of a more neurological nature. As in humans, the congeners 138, 118 and 153 have been found to be amongst the most predominant in brain regions, blood, liver and fat in rats after exposure to A1254 (Kodavanti et al., 1998). After exposure to the less chlorinated A1016, the *ortho*-substituted PCB congeners 17, 47 and 52 were the only congeners detected in the brain of monkeys (Seegal, Bush, & Shain, 1990).

A1254 inhibited transmitter uptake into synapses from rat brain, and both A1254 and A1242 inhibited the uptake of dopamine, glutamate, gamma-aminobutyric acid (GABA) and serotonin (Mariussen & Fonnum, 2001). The mixture also reduced dopamine concentrations in cells in culture and in the developing brain of rats (Bemis & Seegal, 2004).

Both the highly chlorinated A1260 and the less lightly chlorinated A1016 have lead to decreases in brain dopamine concentrations in monkeys up to 44 weeks after a 20 weeks exposure, showing a possible long-term dysfunction in dopaminergic function (Seegal, Bush, & Brosch, 1994). The same mixtures have also produced decreases in striatal dopamine transporter (DAT) and dopamine levels in mice after acute exposure (Richardson & Miller, 2004).

Using the single congener PCB 153 as source of exposure, a decreased dopamine concentration was found in the frontal cortex region of rats (Chu et al., 1996). The hyperactivity in mice after exposure to PCB 77 was found to be associated with decreased dopamine levels and dopamine receptor binding sites (Agrawal et al., 1981). Exposure to the same congener also produced significant elevations in dopamine concentrations in the frontal cortex and of dopamine and its metabolites in the substantia nigra, while exposure to the *ortho*-substituted PCB 47 produced decreased dopamine concentrations in the frontal cortex

and caudate nucleus. Both effects persisted into adulthood (Seegal, Brosch, & Okoniewski, 1997).

Studies using several single congeners suggest that dopamine decrease is only found following exposure to the *ortho*-substituted PCBs, and not for the coplanar, non-*ortho* congeners (Bemis et al., 2004; Mariussen et al., 2001; Seegal et al., 1990).

PCBs have also been found to affect other transmitter systems, long term potentiation (LTP), hormones, cell death and reactive oxygen species (ROS) (Mariussen et al., 2006). For a more extensive review of various neurological effects of PCB, see Mariussen and Fonnum (2006).

Related disorders

Some of the behavioural effects and the consistent findings of decreases in dopamine levels in PCB exposed animals resemble the aetiology of certain disorders. It is therefore natural to draw parallels to disorders found to have a dopamine deficiency.

Parkinson's Disease (PD) is a progressive neurodegenerative disorder affecting about 1% of the population above the age of 55 (Betarbet, Sherer, & Greenamyre, 2002). Patients with the disorder are characterized by tremors in hands and feet, problems with balance and coordination, and they tend to become slower and stiffer with age (Youdim & Riederer, 1997). They have also been found to walk with hunched backs and small steps and have a flat, expressionless face (Brown, Lockwood, & Sonawane, 2005). It is also correlated with psychological problems such as depression and with cognitive impairment and dementia in the later stages (Poewe, 2007; Dubois & Pillon, 1997; Youdim et al., 1997).

Neurochemically, PD is characterized by a profound loss of dopamine neurons in the substantia nigra (Collins & Neafsey, 2002; Youdim et al., 1997). The dopamine explanation of PD has gained its support through medical research on L-dopa and other medicines working on the dopamine system and producing symptom relief (Pahwa et al., 2006; Cools, Barker, Sahakian, & Robbins, 2001; Youdim et al., 1997). Some support for a genetic factor have been found (Gilks et al., 2005; Tanner et al., 1999), and it is assumed that the cause of the disorder is due to a genetic vulnerability with environmental factors such as toxins, insecticides and metals being the triggering cause (Brown et al., 2005; Collins et al., 2002).

Attention Deficit/Hyperactive Disorder (ADHD) is one of today's most prevalent developmental disorders and compared to Parkinson's late onset manifests itself early in childhood (Swanson et al., 1998; Smalley, 1997; Shue & Douglas, 1992). ADHD is found

worldwide (Faraone, Sergeant, Gillberg, & Biederman, 2003) and it is estimated that between 3% to 6% of school children suffer from ADHD (Goldman, Genel, Bezman, & Slanetz, 1998; Cantwell, 1996). The Diagnostic and Statistical Manual of the American Psychiatric Association 4th edition (DSM-IV) (American Psychiatric Association, 1994) define ADHD based on three main symptoms: Hyperactivity, impulsivity and inattention. Further, DSM-IV divides into three subtypes of ADHD: The predominantly hyperactive-impulsive type (ADHD-PH), the predominantly inattentive type (ADHD-PI) and the combined type (ADHD-C).

The aetiology of ADHD is not fully understood, but the heritability in ADHD is amongst the highest for any psychiatric disorder, estimates varying between 70 and 90% (Barkley, 2002; Gjone, Stevenson, & Sundet, 1996). Genetic and imaging studies, together with the neuropharmacology of stimulant medication and animal models, strongly support a dopamine deficiency in ADHD (DiMaio, Grizenko, & Joober, 2003; Kirley et al., 2002). It is possible that a hypofunctioning dopaminergic system lies at the core of the disorder resulting in changed basic learning processes. This system will be vulnerable for toxins and chemicals in the environment (Sagvolden, Johansen, Aase, & Russell, 2005).

Another well-known dopamine related disorder is *Schizophrenia*. The disorder is characterized by an increase and not decrease in dopamine levels, or possibly a combination of both (Abi-Dargham, 2004; Davis, Kahn, Ko, & Davidson, 1991). It is also a disorder difficult to discuss in relation to animal studies due to the nature of the symptoms. Schizophrenia is thus not discussed in relation to the present study.

Aims of the study

The present study investigated behavioural effects in rats exposed to one of three *ortho*-substituted PCBs. The PCBs were selected since they have been found to exist in mother's milk. Two highly chlorinated congeners (PCB 153 and PCB 180) and one less chlorinated congener (PCB 52) were used. Behavioural effects were tested using a reinforcement schedule proved reliable for investigating activity level, impulsivity and sustained attention (Sagvolden & Xu, 2008; Sagvolden, 2000).

Due to the design variations and sometimes contradictory results in the literature, no predictions were made regarding the results. Since some studies have failed to find effects altogether a high exposure dose was administered ensure that results would be present if such results do exist.

Methods

Subjects

The experiment started out with 48 male outbreed Wistar Kyoto (WKY/NHsd) rats purchased from Taconic, USA. During the PCB exposure, the rats were under the care of a veterinarian at the Norwegian Defence Research Establishment, Kjeller. At postnatal day (PND) 24 the rats were sent to the University of Oslo for behavioural testing. The rats were experimentally naïve on arrival.

During habituation and shaping, the rats were housed together in twos or threes in cages of 43x27x18 cm. Later they were moved to individual cages. The rats had access to food (Special Diet Services, Witham, Essex, UK) at all times, but were deprived of water for about 22 hours a day after the initial habituation session ensuring sufficient motivation (Sagvolden et al., 2008). During the experimental procedure, they were given water as reinforcers and had free access to water for 45 minutes in the home cage immediately following each day's trial.

The cages were kept in a housing area with a stable temperature of about 22 ^{o}C with lights on from 07.00 to 19.00 hours every day. The experiments were run between 9.00 and 14.00 hours 7 days a week for a period of about 4 weeks.

The study was conducted in agreement with the laws and regulations controlling experiments with live animals in Norway and approved by the Norwegian Animal Research Authority (NARA).

PCB exposure

PCBs 52 (2,2',5,5'-Tetrachlorobiphenyl), 153 (2,2',4,4',5,5'-Hexachlorobiphenyl) and 180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl) were purchased from Patrick Anderson, Department of Chemistry, University of Umeå, Sweden. The PCBs were dissolved in corn oil and the dose of PCB given was 10 mg/kg body weight. Each rat was randomly assigned to one of the treatment groups or the control group and gavage fed three times between postnatal day 10 and 20. Time and route of exposure (gavage) were chosen to best reflect transferral through mother's milk. The administrations were done by a veterinarian.

A number of deaths occurred in all groups after the exposure, most profoundly in the control group which was reduced from 12 to five rats. The deaths can thus not be attributed to the PCB exposure (alone), but may be due to the gavage feeding. The dose given was about 10 ml/kg which is considered the maximum of recommended dose (Brown, Dinger, & Levine, 2000). At the start of the experiment there were n=10 for the PCB 52 group, n=6 for the PCB 153 group, n=9 for the PCB 180 group and n=5 in the control group, making the total number of rats 30.

Behavioural apparatus

The cages used for the experiments were Campden Instrument Operant Chambers for rodents with a working space of 25x26x30 cm in lab 1 and 25x24x20 cm in lab 2. A light of 2.8 W situated in the ceiling of the chamber, and a fan ensuring air circulation and masking noise was on during the entire course of the experiment. Each chamber was placed in a light- and sound resistant outer housing (Campden). Eight chambers were in use in lab 1 and seven in lab 2.

Each chamber was equipped with two retractable levers. Above each lever a 2.8 W cue light indicated the correct or wrong lever (response). Responses were measured when the rat pressed the lever with 3 grams of dead weight and activated a micro switch. A liquid dipper delivered a drop of tap water (0,01 ml) as a reinforcer when activated.

The dipper was housed in a small cubicle separated from the cage by a transparent plastic flap of 7x5 cm, easily opened by nose or paw. The cubicle was also equipped with a cue light (2.8 W) that got lit every time a reinforcer was presented. The reinforcer was available for 3 s after opening of the flap. If not collected within 5 s after the activation, the dipper was lowered to its initial position and the light was turned off.

The rats divided on the two available labs and assigned a cage and time of testing, divided on the two available behavioural labs. This was done in a randomized and balanced fashion regarding the PCB/corn exposure to minimize any errors on behalf of the time of running and cage numbers.

The animals' behaviour was recorded by a computer programme, LabVIEW 7.1 (National Instruments, 2004), which also scheduled the lights and the delivery of the reinforcers.

Procedure

Habituation, magazine training and shaping

All the habituation, magazine training and shaping was done in lab 1with the fan and ceiling light turned on.

The habituation training was done the day after arrival at the lab, making it PND 25. The flap was taped open, no water was delivered, and both levers were retracted. None of the cue lights were on at any time. The rats were in the cages for about 20 min.

The following day the rats went through two sessions of 30 minutes magazine training. Both levers were retracted, none of the cue lights above them lit, and the plastic flap was taped open giving free access to the water cubicle. A variable schedule was used, and independent of the rat's behaviour a drop of water was delivered on the average every 10 s. The cubicle's cue light was lit with each water delivery.

The next two sessions, the tape was removed and the flap was in its initial position requiring the rat to open it to get access to the water. Water was delivered each time the rat successfully opened the flap, an action also activating the cue light in the cubicle. The levers were retracted at all times and none of the cue lights were lit. The rat was left in the cage until it had fully learned the procedure. Both sessions were conducted on the same day, one in the morning and one later in the afternoon.

The response shaping began on PND 28 with the left lever present and the cue light above it lit at all times. The right lever was retracted and its light turned off. Every time the lever was pressed, the response triggered the delivery of a reinforcer in the water cubicle accompanied by the cubicle's cue light. Lever pressing was hand shaped according to the method of successive approximation (Catania, 2007). Each rat was monitored to make sure the procedure was learned and then left in the age for an additional 15 min.

The next day the same procedure was followed for shaping of right lever presses, with the left lever retracted and only the light above the right lever lit.

Training schedule

After successfully finishing the shaping procedure, the rats started training on a frequent reinforcement schedule. From now on both levers were present at all times. Whether the left or right cue light was lit varied randomly. Pressing the lever under the light was considered a

correct response and produced a reinforcer (water). When water was presented, the cue light in the cubicle was turned on. The ceiling light and fan was on during the entire session.

The programme lasted 30 min, and a Fixed Interval (FI) schedule of 3 s (FI 3s) was used, scheduling a reinforcer for every correct response (due to the 3 s availability of the reinforcer). Pressing the lever without light had no consequences, - hence there was a concurrent Extinction (EXT) schedule, making this a multiple Fixed Interval/Extinction schedule (mult. FI EXT). No external stimuli signalled time since last response or when a reinforcer was programmed according to the schedule. The training programme was run for five days marking session -5 to -1 and the rats were run at the same time and in the same cage each trial.

Main schedule

The initial 5 sessions on the training programme were succeeded by the main schedule of infrequent reinforcement. Here, a Random Interval (RI) schedule was used providing reinforcers at an average of every 3 min (RI 180s). On the wrong lever the extinction schedule operated, thus making this a multiple Random Interval/Extinction schedule (mult. RI EXT). As on the training programme, the animals were given no indications as to when a reinforcer was programmed or how much time had passed since the last response.

This programme initially lasted for 90 min. Due to computer problems in lab 2, all rats had to be run in lab 1 after the first week (i.e. starting on session 7) making it necessary to reduce the time of testing to 45 min to be able to run all rats each day. The main programme was run for 21 days, marking sessions 0 to 20. Each rat was run at the same time and in the same cages each trial (apart from when the rats from lab 2 were moved to lab 1).

Behavioural measures

Time, produced and collected reinforcers, number of flaps, and correct and incorrect lever presses were recorded.

Activity was measured by the total number of lever presses, on correct and wrong levers combined. A large increase in activity can be seen as a measure of hyperactivity.

Inter response times (IRTs), the time passing between two lever presses, were divided into short IRTs (<0,67s) and long IRTs (>0,67s). Responses closer than 0,67s in time will never produce a reinforcer, and can be seen as an inability to respond in a reflected and

planned manner, a characteristic of impulsive behaviour (Johansen, Aase, Meyer, & Sagvolden, 2002). The number of short IRTs can thus be seen as a measure of impulsivity.

The numbers of correct lever presses were divided by the total number of presses giving a measure of percentage correct responses. Sustained attention means that a stimulus controls behaviour over a longer amount of time (Catania, 2007). As the main schedule produce reinforcers seldom and in an infrequent manner, sustained attention will be necessary to produce a correct responses based on whether the right or left cue light is lit.

Data Analysis

As a result of computer problems in lab 2, some of the data from the first sessions were lost. A couple of the cages did on a few occasions fail to record flap openings and hence produced too many reinforcers. Data for the rats in those cages were excluded for the sessions in question. Missing data was substituted by calculating the means of the preceding and following sessions. Other outliers were removed using a method removing data exceeding two standard deviations from the group means. One rat was excluded from the PCB 52 group on account of consistent deviant behaviour.

The first five sessions (i.e. session -5 to -1), when run on the training schedule, were analyzed separately. For the main schedule, the last two weeks, i.e. session 7 to 20, were used as they mark the sessions where the rats showed more stable state behaviour after the transition from the training programme. All measures were cumulative and the analyses were done on the end result of each session.

The analyses conducted were repeated measures analyses of variance (ANOVAs). The within-subject factor was the sessions and the between-subjects factor the treatment groups. All analyses were done in Statistica 6.0 (StatSoft, 2001), with the previous calculations and processing executed in Excel (Microsoft, 2003) and SPSS 14.0 (SPSS, 2005).

Results

General

All the rats learned the magazine training and shaping and progressed onto the training programme and further onto the main programme.

The rats showed overall higher activity levels after transition to the main schedule. While all groups produced about a 100 lever presses when frequent reinforcers were given, this increased from about 400-600 in the beginning of the main schedule till a peak at 600-1000 around sessions 5-7 before starting a decline and ending at about 350-650 presses depending on the groups (Fig. 2).

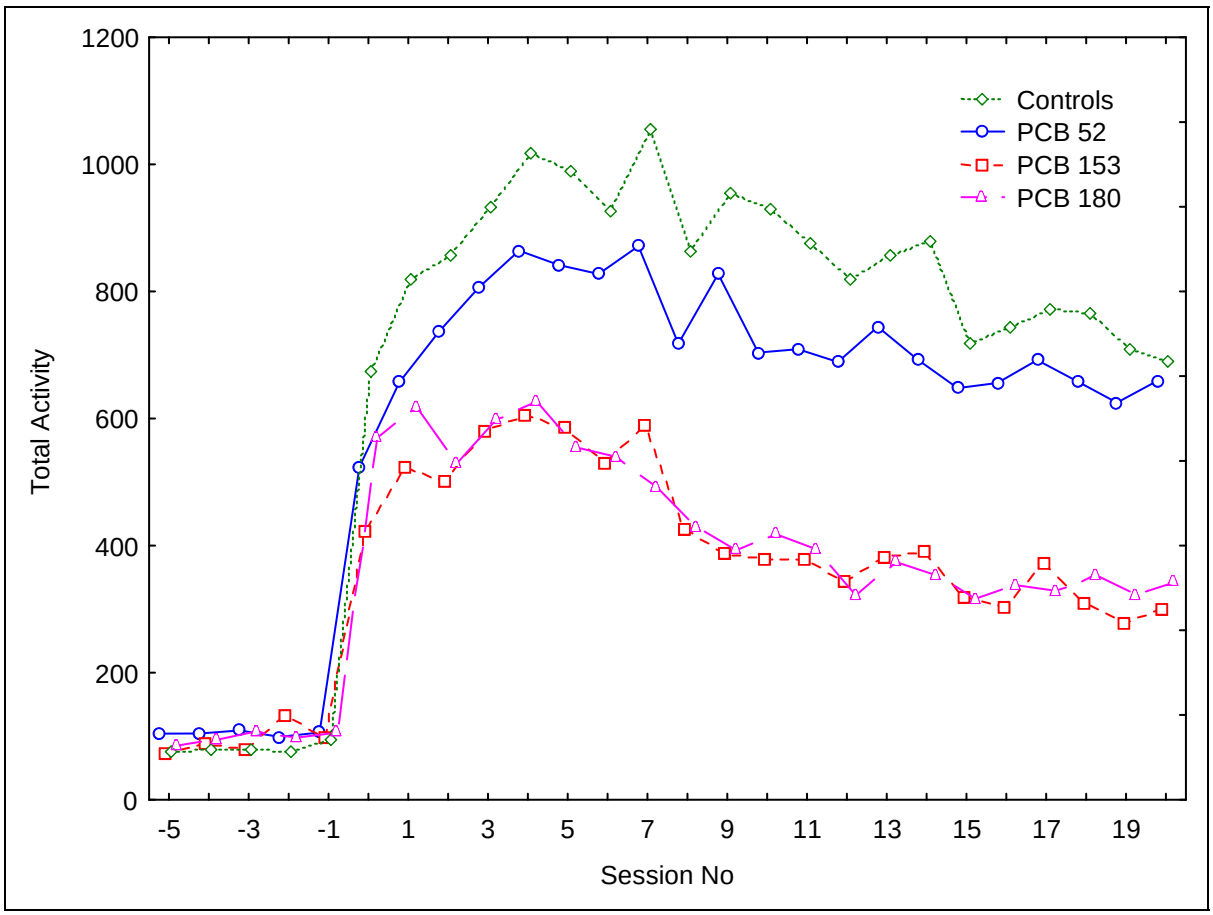

Figure 2 Total activity across sessions

As with total activity, the numbers of short IRTs increased after transition to the main programme. From almost zero short IRTs registered on the training programme for all groups, this increased to ranging from about 20 to 60 depending on group on the main programme (Fig. 3).

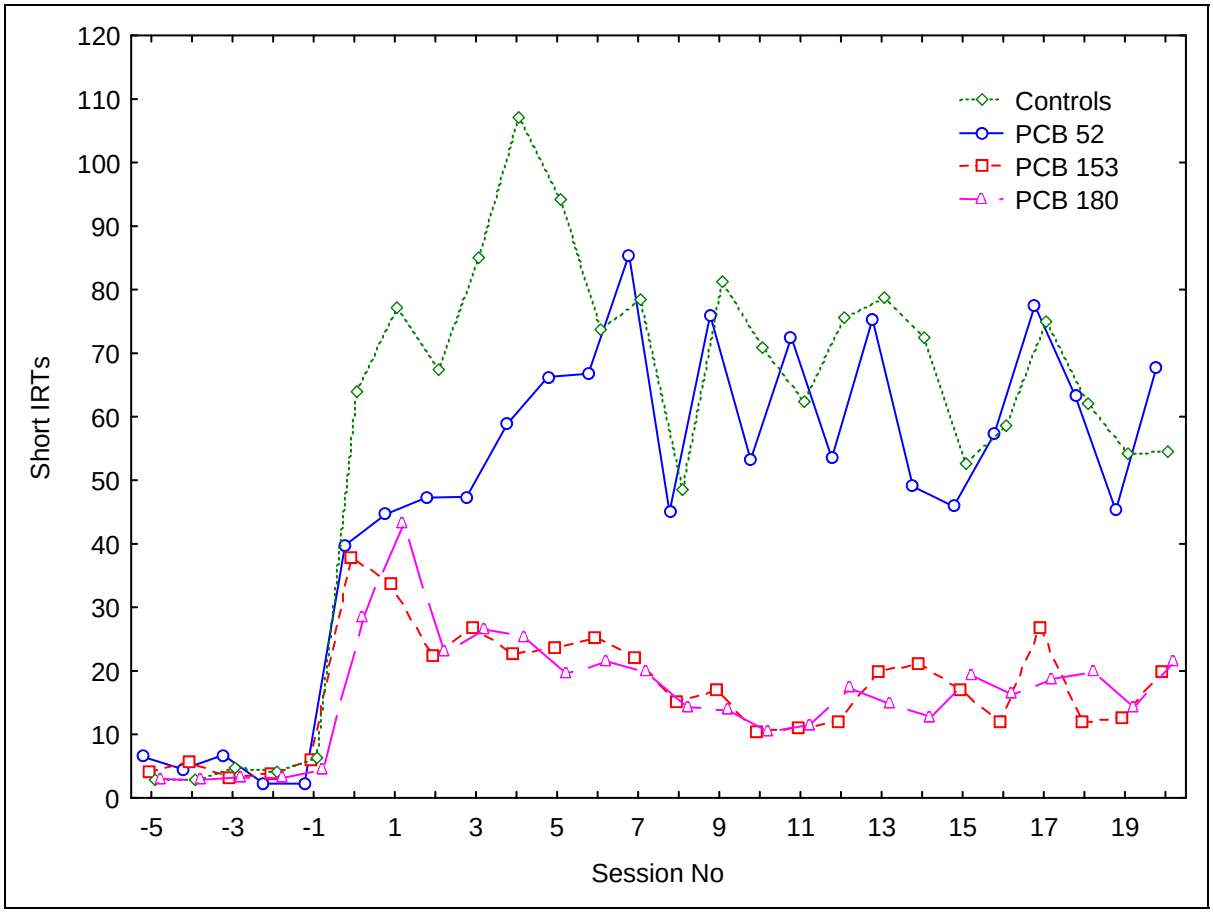

Figure 3 Total number of short IRTs across sessions

The percentage of correct responses had a steady progress during the training programme and all rats ended up with a score of above 80% correct responses making it clear that they had acquired the procedure and were ready to start on the main schedule. The transition to the main programme manifested itself with a clear drop in the percentage of correct responses in all groups, to between 60 and 70%. Towards the final sessions there appear to be a slight improvement (Fig 4).

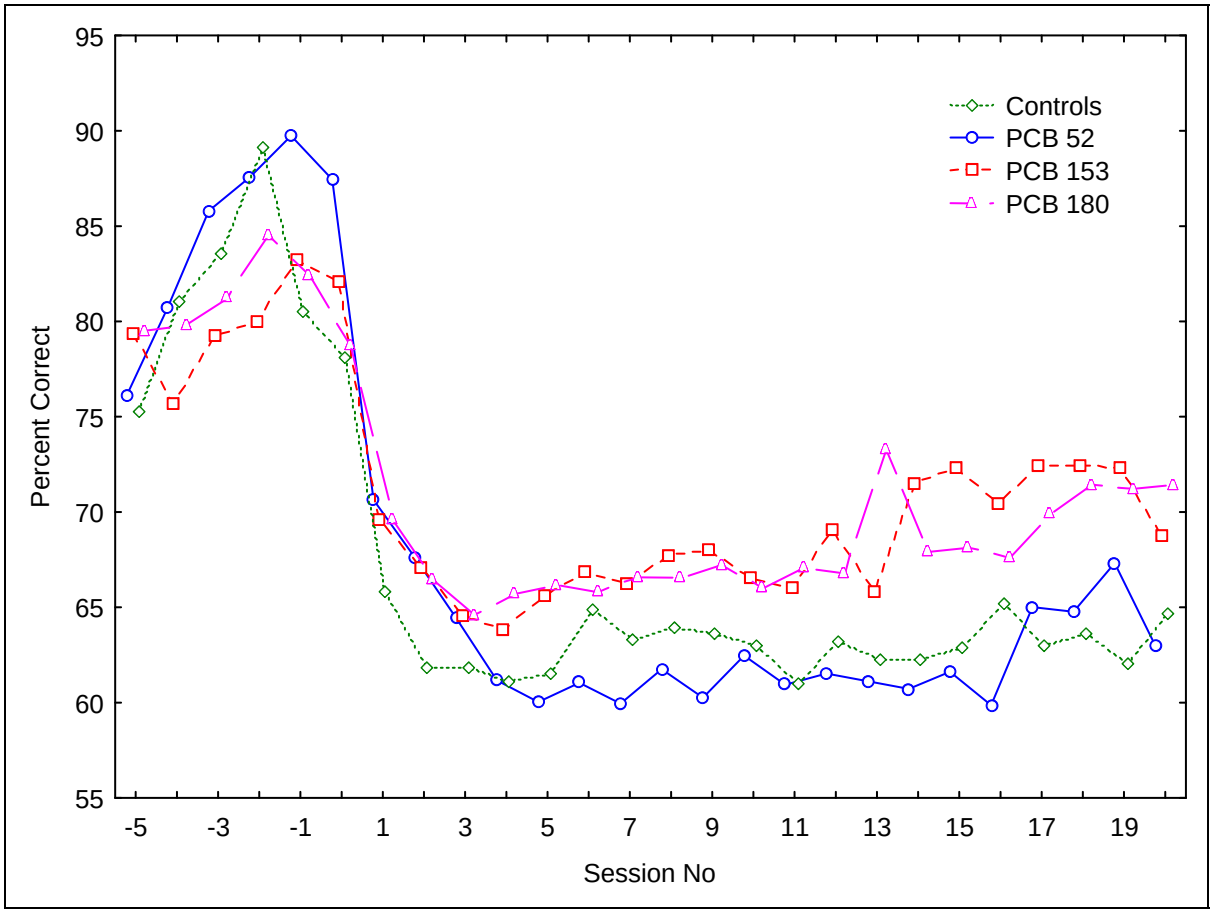

Figure 4 Percent correct responses across sessions

Training

There were no significant group differences on the training programme on any of the measures (the ANOVA showing all *p*s > 0.13 on main effect of group).

All the rats did improve over the course of the programme. The ANOVA showed a main effect of session, F(4,92) = 5.29; p = 0.001 on the percent of correct presses, F(4,96) = 4.268; p = 0.003 for produced reinforcers and F(4,96) = 5.465; p = 0.001 for collected reinforcers. There were no main effects of session on the other measures.

For the short IRTs an interaction effect was found, the ANOVA showing F(12, 88) = 2,5179, p = 0.001. No interaction effect was found for the other variables.

Main schedule

Significant group differences were found on the main variables: Activity, impulsivity and sustained attention, on the main programme (Fig 5).

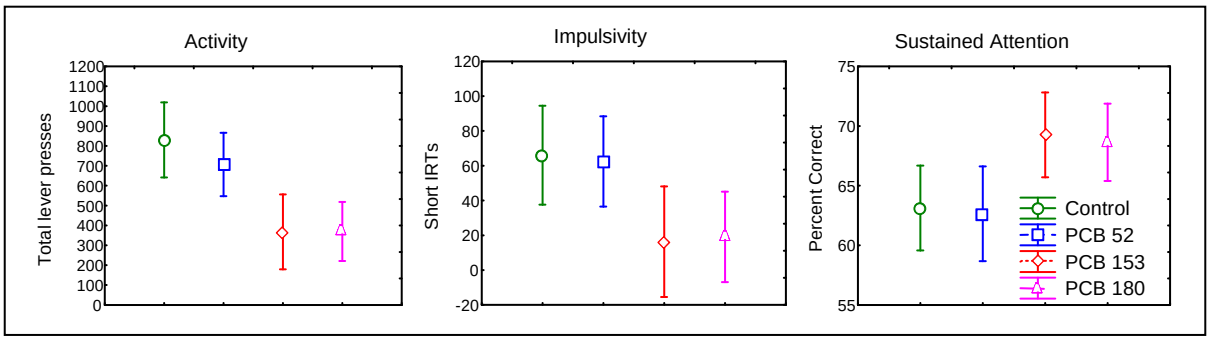

Figure 5 Group means on main schedule ± 95% confidence interval

Activity

The PCB 152 group and the PCB 180 group differed from the controls and PCB 52 group in total activity, with the latter two groups being the most active. The difference was significant on the group level, the ANOVA showing a main effect for group, $F(3,21) = 8.05$; $p = 0.001$. Overall the activity level of rats exposed to PCBs 153 and 180 was about half that of the PCB 52 group and the controls.

There was a decline in activity in all groups toward the end of the study. The ANOVA showed a main effect for session, $F(13,273) = 2.138$; $p = 0.000$. No interaction effect was found.

Impulsivity

The PCB 153 group and PCB 180 group showed less impulsive behaviour than the PCB 52 group and the controls. The ANOVA showed a main effect of group, $F(3,17) = 4.111$ $p = 0.023$. No main effect for session or interaction effect was found on this measure.

Sustained attention

A group difference was seen between the controls and PCB 52 group and the PCB 153 group and PCB 180 group with the latter two having overall higher scores. The ANOVA showed a main effect of group $F(3,16) = 4.284$; $p = 0.021$.

Towards the end of the experiment the rats did improve, and the measure of percent correct showed a main effect of session, the ANOVA showing $F(13,208) = 2.138$; $p = 0.013$. No interaction effect was found.

The ANOVA showed no significant group effects on the number of produced and collected reinforcers or visits to the water cubicle (flaps). However, a main effect of session was found on the number of flaps, the ANOVA showing $F(13,234) = 4.719$; $p = 0.000$. No interaction effects were found.

Discussion

This study investigated behavioural effects of exposure to three di-*ortho*-substituted PCBs. The rats exposed to the two congeners PCB 153 and PCB 180 showed significantly less overall activity and impulsive responding than the PCB 52 group and controls. There was also a difference between the same groups on the sustained attention measure, with the PCB 153 and PCB 180 groups scoring significantly higher.

Despite all three congeners used in this study being di-*ortho*-substituted, and thus structural similar, behavioural effects were only found for PCB 153 and PCB 180, not for PCB 52. In a study of mice, Eriksson and colleagues found behavioural changes in mice given a dose of 4.0 mg/kg body weight of PCB 52 (Eriksson et al., 2006). The time of exposure was similar to that in this study, but the mice were tested at a later age (4 and 6 months). They were also tested using a different design than the reinforcement schedule used here which may account for the discrepant results.

PCB 52 is a less chlorinated congener than PCB 153 and 180, having only four chlorine substitutions. It has been suggested that the less chlorinated PCBs are less toxic due to being more soluble and thus less biological stable (Roegge et al., 2000; Giesy et al., 1998). In a study exposing rats to the mixture A1254, PCB 153 and 180 were found in the rat brain on dissection, while the PCB 52 could not be detected despite also existing in the mixture (Kodavanti et al., 1998). PCB 52 is also found to have lower prevalence in humans than the other two (Korrick et al., 2000). A possible explanation for the lack of behavioural effects found in the PCB 52 group might therefore be that the congener, due to being less chlorinated, to a smaller degree is stored in the brain and nervous system. As neurological

assessment of the rats in this study was not possible a definite conclusion on this matter can not be drawn.

Previous studies have shown that PCB exposure produce increased activity and impulsivity and impaired sustained attention in designs rather similar to this study (Berger et al., 2001; Holene et al., 1998). The results for the PCB 153 and PCB 180 groups are thus contradictory to the previous findings. Hypoactivity has, however, also been found in rats given high doses of A1254 (Nishida et al., 1997). Facilitating effects have been found in some studies (Schantz et al., 1996; Schantz et al., 1989), but as these measured learning and working memory, rather than attention, one should be careful to draw any parallels. In two studies measuring both activity and attentional levels Bushnell and colleagues failed to find any effect of PCB exposure at all (Bushnell et al., 2002; Bushnell et al., 1999). Motor difficulties (see below) may explain the facilitatory effect found in the PCB 153 and PCB 180 group. If the body is more rigid or characterized by a lack in balance or coordination, every response will costs more. Each response will therefore be more demanding, and this can lead to the rats being reluctant to explore and use unnecessary energy on trying out responses.

The dose used in this study was high. This may explain why they became hypocactive rather than hyperactive as animals have been in other studies. For example, the dose used in the Holene and colleagues study is smaller than in the present study, as the rats were exposed only through breast milk of dams given 5 mg/kg bodyweight PCB 153 or 126 (Holene et al., 1998). The doses in the Berger and Bushnell studies were also lower (Bushnell et al., 2002; Berger et al., 2001; Bushnell et al., 1999). Exposure to A1254 have produced dose-dependent hypoactivity in rats, – the larger the dose of exposure, the more hypoactive the rats became (Nishida et al., 1997). In rats given a chronic exposure, the no-observable effect level (NOEL) was 10 mg/kg bodyweight rat. The rats were adult at time of exposure, and one can speculate that the NOEL would have been lower had they been exposed at a younger age, as in the present study. The developing nervous system appears to be more sensitive to toxins when there is a rapid growth of the brain. In rodents this period is during the first 3-4 weeks after birth (Kodavanti, 2005).

Dopamine transporter levels have also been found to show a decrease in a dose-dependent manner in mice after exposure to an environmentally relevant mixture of PCBs (Caudle et al., 2006). An association between dopamine levels and behavioural measures has been found after PCB exposure (Agrawal et al., 1981). Together with behaviour results from this study and studies using smaller PCB doses, this might suggest a possible inverted U-shape on activity levels, with small doses causing hyperactivity and higher doses causing

hypoactivity. Doses so low that they fail to affect the nervous system will show no or little behavioural effects.

The hypoactivity produced in this study might indicate motor difficulties. Motor problems have also been found in PCB exposed mice, similar to the so-called spinners (Agrawal et al., 1981; Chou, Miike, Payne, & Davis, 1979). In addition to increased activity doing circular movements, spinners tend to wander aimlessly around while shaking the head (Chou et al., 1979). Motor disturbances such as shaking are one of the core symptoms of Parkinson's Disease (Youdim et al., 1997). People suffering from the disorder also tend to get rigid and thus immobile with age (Youdim et al., 1997). As mentioned, motor problems may subsequently inhibit the rats' movement and decrease the activity level.

PD is characterized by a decrease in dopamine (Collins et al., 2002; Cools et al., 2001; Youdim et al., 1997). The same decrease has also been consistently found in PCB exposed animals, also for the single congener PCB 153 used in this study (Chu et al., 1996). In healthy people, the dopamine level decreases over the years, but in PD this decrease is accelerated (Youdim et al., 1997). It has also been suggested that PCBs lead to a premature neurological aging process of the brain (Piedrafita et al., 2008; Eriksson et al., 2006). The premature aging process may thus explain both PD and the neurological and behavioural changes in PCB exposed animals. Direct associations between PCB exposure and the disease have also been found. Both PCB 153 and PCB 180 have been found in larger amounts in the caudate nucleus of PD patients than in controls (Corrigan, Murray, Wyatt, & Shore, 1998). In a retrospective study of PCB-exposed workers, higher prevalence of PD and dementia was found among highly exposed women (Steenland et al., 2006).

Holene and colleagues (Holene et al., 1998) found male rats exposed to PCB 153 to show hyperactivity, impulsivity and problems with attention similar to that of the Spontaneous Hypertensive Rat (SHR), a well known animal model of ADHD (Sagvolden, 2000). The SHR show increased numbers of lever presses and short IRTs and a lower percent of correct responses on reinforcement schedules similar to that in this study (Sagvolden, 2000). Similar findings were found in rats exposed to A1248 or PCB-contaminated St. Lawrence River carp (Berger et al., 2001). Findings from epidemiological studies on humans also indicate an association between PCB exposure and ADHD, with children showing increased activity levels, impulsivity and cognitive problems (Stewart et al., 2005; Jacobson et al., 2003; Grandjean et al., 2001; Chen et al., 1994). As with PD, this association is further strengthened by the similarities in dopamine decreases.

Since the sustained attention measure is not negatively affected, it is difficult draw a direct connection to the predominantly inattentive or combination type of ADHD. It is presumed that the facilitatory effect found can be a result of motor problems rather than resulting from cognitive changes. This makes it further problematic to draw any conclusions on the attentional measure in this study and its relations to the impairments found in ADHD. This study is thus only suggestive of a PCB effect on the hyperactivity symptom of ADHD, though it due to its high dose of exposure, failed to produce such an increase in activity.

The aetiology of ADHD is not fully understood, but one possible explanation may be that some people have a genetic vulnerability for developing the disorder and that toxins like PCB can contribute to the development of the disorder through influence on the dopaminergic system (Sagvolden et al., 2005). This may explain why increased hyperactivity and other ADHD like symptoms have been observed in areas with high PCB exposure. A similar aetiology, with a genetic vulnerability factor, is suggested for PD (Brown et al., 2005; Collins et al., 2002). Further research on animal models of the disorders exposed to PCBs should be conducted to investigate possible triggering effects.

A possible explanation of the results in this study can thus be that higher doses of PCB exposure causes Parkinson-like rather than ADHD-like symptoms and that the lack in activity is caused by motor deficiencies. The behavioural symptoms of PD usually occur when there is an 80% decrease in dopamine levels (Betarbet et al., 2002). ADHD patients do not show such motor problems, and one can thus speculate that the dopamine deficiency in this disorder is smaller than in PD. Both behavioural and neurological findings after PCB exposure have been found to vary according to dose of exposure. As this study only used one dose of exposure one cannot draw any conclusions on this subject, and further research into dose-response curves are needed. Also, the rats were tested in a design well used to detect activity, impulsivity and sustained attention, a method well equipped to identify animal models of ADHD, but less so for PD. As similar tests have not been conducted on animal models of PD, conclusions are therefore difficult to verify.

Together with previous studies on PCBs, this study further strengthens the fact that this toxin is of harm to humans and our environment. Today there is a production ban on the chemical, but its effect on behavioural effects calls for caution in dealing with residue and food possibly being contaminated with PCBs.

Methodological limitations

The animals in this study were subjected to single congener exposure. The exposure in the true natural environment consists of several different congeners, and a single congener approach might therefore fail to reflect the complex mixtures found in humans and a possible interaction effect between these congeners (Bowers et al., 2004). However, given the inconsistency in the literature, one should start by assessing simple effects and then later move on to interaction effects. As long as the congeners used are of relevance for human exposure, a single congener approach can thus be defended (Ulbrich et al., 2004).

Due to unexpected loss of animals before the start of the study, the control group was smaller than planned, only counting five animals. A small control group raises concerns as to how well the animals are representative of a "normal" group. Therefore, seven extra controls arrived at the lab one week after the arrival of the initial 30 rats. These had not been PCB exposed or gavage fed corn oil. They were run as extra controls, going through the same programmes as the original rats, though starting a week later. It was, however, decided to keep them out of the final design as they were not gavage fed and started the experiment one week later, opening for third variables. When included as part of the control group though, the results still pointed in the same directions as when only the five original controls were included. A similar trend for a group difference between the PCB 153 and PCB 180 groups and the PCB 52 group and controls was seen on the attentional measure, this did however fail to attain significance (results not shown). On the activity and impulsivity measures, significant differences were found between the groups in concordance with the results using the five original controls (results not shown). This might indicate that the control group, though small in size, well represent a non-exposed group.

As they were less active overall, one can assume that the PCB 153 and 180 exposed rats would produce fewer numbers of IRTs, – long as well as short. The impulsivity measure may thus only further reflect the hypoactivity. Impulsivity has therefore not been treated separately in this discussion, but as part of the behavioural changes in activity level. However, while the total activity measure showed a decrease towards the end of the study, this was not the case for the short IRTs. This might suggest that the impulsivity measure reflects more than just the activity level.

Some studies indicate that there are sex differences in rodents after PCB exposure e.g. (Holene et al., 1998; Schantz, 1995). There might therefore be a chance that the results in this study could have been different using a female sample.

Conclusion

Rats exposed to PCB 153 or PCB 180 showed significantly less total activity and impulsivity compared to a control group and rats exposed to PCB 52. The two highly chlorinated PCB congeners also scored higher on the sustained attention measure. A possible explanation for this is that the high dose given in this study influenced behaviour through causing motor problems. Together with contradicting results from other studies, this raises a need for doing further studies investigating dose-response curves. It may also be that PCBs by altering dopamine levels can be a contributing factor in the development of Attention Deficit/Hyperactivity Disorder and Parkinson's Disease. Studies investigating a possible vulnerability factor in these disorders using animal models should therefore be conducted.

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