Developmental Exposure of Rats to a Reconstituted PCB Mixture or Aroclor 1254: Effects on Long-Term Potentiation and [3H]MK-801 Binding in Occipital Cortex and Hippocampus

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The central nervous system is one of the target organs for polychlorinated biphenyls (PCBs). We measured the effects of maternal exposure of Long-Evans rats to a mixture of PCB congeners reconstituted according to the pattern found in human breast milk (reconstituted mixture, RM) on long-term potentiation (LTP) in two brain regions. Exposure of the dams via food started 50 days prior to mating and was terminated at birth. In the first experiment, adult male and female offspring were exposed maternally to 40 mg/kg of the RM or the commercial mixture Aroclor 1254 (A1254). LTP and paired-pulse inhibition were measured in slices of the visual cortex. In addition, the binding of [3H]MK-801 to the N-methyl-D-aspartate (NMDA) receptor-ion channel as well as the [3H]muscimol binding to the GABA-A receptor in membrane preparations from the occipital cortex and hippocampus were determined. LTP as well as [3H]MK-801 binding were significantly reduced in the cortex following PCB exposure, while [3H]MK-801 binding in the hippocampus was not affected. In a succeeding experiment, LTP was determined in cortical and hippocampal slices from rats at postnatal days 10 to 20, following exposure to 0, 5, or 40 mg/kg of the RM. Cortical LTP was significantly affected by the RM while no effects were seen in hippocampal LTP. Taking the two experiments together, PCB exposure significantly reduced LTP, as well as [3H]MK-801 binding, in the cortex and had no effect in the hippocampus. The LTP deficits can only partly be related to the reduction of binding sites to the NMDA receptor; other PCB-induced neurochemical changes have to be assumed.

Key Words: polychlorinated biphenyls; NMDA receptor; long-term potentiation; visual cortex; hippocampus; brain slices.

Polychlorinated biphenyls (PCBs) are persistent environmental pollutants that accumulate in food chains (Clarkson, 1995) and are found at elevated levels in human milk (Dewailly et al., 1996). Although production of PCBs has stopped in most countries, they persist in the environment due to their chemical and thermal stability. PCBs are known to be neurotoxic, inducing neurochemical and behavioral disruptions in humans and experimental animals (for review: Brouwer et al., 1995). Developing individuals appear to be particularly susceptible, and the early developmental exposure period is most important for the induction of neurobehavioral disorders (Lilienthal and Winneke, 1991).

Several epidemiological studies conducted in infants and children have reported possible associations between a poorer cognitive and psychomotor development and perinatal exposure to PCB mixtures at contemporary background levels (Huisman et al., 1995; Jacobson and Jacobson, 1996). In neonates, significant relationships were found between prenatal PCB exposure and performance impairments using the Neonatal Behavioral Assessment Scale (Stewart et al., 2000). Deficits in performance on a visual recognition memory task at 7 months of age were reported (Jacobson et al., 1985). Cognitive/motor deficits were found among children who had accidental perinatal exposure to PCBs in cooking oil (Chen et al., 1994; Rogan and Gladen, 1992). Recently, negative associations were reported between PCB concentrations in breast milk and mental development in 7-month-old children (Winneke et al., 1998).

In primates and rodents, perinatal PCB exposure resulted in neurobehavioral effects: impairments of cognitive tasks, indicative of effects on learning and memory, were reported in monkey offspring following exposure to PCB mixtures (Levin et al., 1988; Schantz et al., 1989). PCB exposure of rodents during development can adversely affect later cognitive and behavioral functions such as impaired acquisition of one-way avoidance learning, depressed visual placement, and increased locomotor activity (Lilienthal et al., 1990; Lilienthal and Winnike, 1991; Tilson et al., 1979). In general, the results of animal studies tend to show that PCBs produce developmental changes in behavioral functioning that are consistent with the results reported in humans (Tilson et al., 1990).
Information is lacking, however, about which of the brain structures could be affected by developmental PCB exposure and thus contribute to these long-lasting behavioral changes. Neuronal plasticity is the underlying property of the nervous system that enables it to adapt to various stimuli during development and adulthood. It is the basis for long-term electrophysiological changes in synaptic transmission, learning processes as well as regeneration. Long-term potentiation (LTP) is a persistent synaptic enhancement induced by high-frequency stimulation of afferents (Bliss and Lømo, 1973) and has been suggested to be an important component of the cellular basis of certain forms of memory and learning (Collingridge 1987; Lynch and Baudry, 1984). It can be measured in different brain regions, including the hippocampus and the neocortex (Artola and Singer, 1987; Kirkwood et al., 1993; Lee 1982) and it was suggested that it might be a model of processes of functional plasticity in cortical regions (Kato et al., 1991; Perkins and Teyle, 1988). LTP has been introduced in neurotoxicological studies on different environmental pollutants (e.g., Altmann et al., 1993, 1994, 1995; Gilbert et al., 1996) and has proven to be a useful tool for linking behavioral and neurochemical outcomes.

In previous studies, our group showed that maternal exposure of rats to a single coplanar PCB congener changed LTP, measured ex vivo, in slices from the visual cortex of adult offspring, whereas no changes were seen in hippocampal slices (Altmann et al., 1995). Furthermore, the effects of prenatal exposure to a coplanar PCB congener were compared with those to an ortho-chlorinated PCB congener of the same dose on LTP, measured ex vivo, in different regions of the developing (postnatal days 11 to 19) brain (Altmann et al., 1998). Again, the coplanar PCB altered LTP in visual cortex, while in hippocampal CA1, LTP did not differ from controls. Recently it was reported that developmental exposure of rats to the commercial mixture Aroclor 1254 (A1254) resulted in LTP impairment in hippocampal dentate gyrus measured in vivo (Gilbert and Crofton, 1999; Gilbert et al., 2000).

To date, most studies have used either single PCB congeners or commercial mixtures of PCBs to evaluate neurotoxic effects. In the present study, we wanted to more closely model the human environmental exposure conditions and so used a reconstituted PCB mixture (RM), which was composed according to the congener-pattern found in human breast milk. Since, in our previous studies, only cortical functions were affected by PCBs (Altmann et al., 1995, 1998), we measured LTP, in the first experiment, in slices of the visual cortex from adult offspring, following maternal exposure to the RM compared to the commercial mixture A1254. In addition, paired-pulse stimulation was applied in order to investigate the balance of excitation and inhibition in the cortical region. By testing male as well as female rats, we assessed possible sex-dependent differences in PCB effects. Furthermore, in order to clarify whether PCB-induced LTP deficits could be explained by changes of the NMDA receptor, we measured [³H]MK-801 binding in cortical and hippocampal tissue of littermates. Additionally, possible effects on the inhibitory neuronal circuitry were determined by measuring [³H]muscimol binding in cortical and hippocampal preparations of the same animals. Following the outcome of the first experiment, in the second experiment, LTP was measured in visual cortex as well as in hippocampal CA1 in slices of rat pups exposed maternally to two different concentrations of the RM. This is the first study that investigates the effects of RM on LTP and [³H]MK-801 binding in occipital cortex using identical exposure conditions. A preliminary account of some of these findings has appeared in abstract form (Altmann and Lilienthal, 1999; Lilienthal et al., 1998).

MATERIALS AND METHODS

Experimental animals and exposure. For the experimental protocols, the current version of the German law on the Protection of Animals was followed. Inbred Long-Evans rats of both sexes (108 females and 54 males in Experiment 1, 104 females and 52 males in Experiment 2) were obtained from Mollegård, Denmark, at about 40 days of age. The females were assigned at random to the experimental groups. Until mating, all animals were housed, by groups of 4, in standard macroleone cages lined with wood shavings. A 12-h:12-h light-dark cycle was kept with lights on at 0600 hours. The temperature was maintained at 23°C and the relative humidity was in the range of 55–60%. Food and water were freely available to the animals. The measurements described herein were part of a larger study, which also included several behavioral tests.

Experiment 1: Adult animals. A PCB mixture, reconstituted according to the pattern found in human breast milk (RM), was compared with the commercial mixture Aroclor 1254 (A1254). The RM consisted of the following 14 different congeners (IU PAC-numbers, proportions in mixture are given in parentheses): PCB-118 (73 mg/g), PCB-138 (221 mg/g), PCB-146 (31 mg/g), PCB-153 (276 mg/g), PCB-156 (38 mg/g), PCB-170 (74 mg/g), PCB-180 (140 mg/g), PCB-187 (48 mg/g), PCB-28 (59 mg/g), PCB-105 (25 mg/g), PCB-101 (14 mg/g), PCB-77 (18 µg/g), PCB-126 (83 µg/g), PCB-169 (37 µg/g). The relative proportion of the 14 congeners in the RM was derived from the literature (Duarte-Davidson et al., 1992; Jensen, 1991; Noren and Lunden, 1991; Safe et al., 1985; Schulte and Malisch, 1984). Together they account for about 80–85% of the total PCB content measured in human serum and adipose tissue, according to recent reports (Luotamo et al., 1991; Stellman et al., 1998).

Three groups of female Long-Evans rats were exposed via food to RM, A1254, or the vehicle (controls). Food pellets containing 40 mg/kg of the respective PCB mixture dissolved in olive oil were made by the manufacturer (Ssniff, Soest, Germany). This PCB dosage resulted in an estimated average daily intake of about 4 mg PCB/kg body weight for an average female weighing 200 g and an average diet intake of 20 g/day. The food for the control group contained only the vehicle olive oil. Fifty days after the start of exposure, females were mated with hitherto unexposed males for 10 days. Exposure was continued throughout mating and gestation until termination at parturition, postnatal day 0 (PND 0). Due to transfer of PCBs via dams’ milk, exposure of the offspring continued until weaning. Adult male and female offspring were tested at PND 180 to 220. The mean age did not differ between exposure groups, because the order of testing was balanced with respect to age. Only one male or one female per litter was used. Females were taken on the time of diestrous for electrophysiological recordings. LTP and paired-pulse potentiation/inhibition were measured in cortical slices from 13 males and 10 females per exposure group. For receptor-binding studies, the hippocampus and occipital cortex of male littermates were dissected on ice and stored at −80°C. The electrophysiological as well as the binding experiments were performed as a blind study with respect to exposure condition.

Experiment 2: Developing animals. Three groups of dams were fed diets with 0, 5, or 40 mg RM/kg (controls, 5 mg RM, or 40 mg RM). The exposure...
procedures were identical to Experiment 1. The PCB concentrations in the food resulted in an average daily PCB intake of 0.5 mg/kg body weight (5 mg RM) or 4 mg/kg body weight (40 mg RM), respectively, of the dams. Because male offspring were used for neurobehavioral tests (e.g., passive avoidance learning, conditioned place preference, sweet preference, see Lilienthal et al., 2000), only female offspring were tested. One animal per litter was used on PNDs 10 to 20. The mean age did not differ among exposure groups, because the order of testing was balanced with respect to age. Cortical and hippocampal slices were prepared from a total of 28 pups (controls: n = 9; RM-5 mg: n = 9; RM-40 mg: n = 10). The investigation was performed as a blind study.

**Slice preparation and recording.** The rats were decapitated under light ether anesthesia and the brains quickly removed and immersed in cold (3–5°C) oxygenated (95% O2, 5% CO2) artificial cerebrospinal fluid (ACSF). In Experiment 2, one hemisphere was used for cortical, the other for hippocampal slices. The slices were cut using a vibratome (Vibracut 1.4, FTB) and incubated at 32°C for at least 1 h in oxygenated ACSF. The ACSF was composed of (in mM): NaCl 124, KCl 5, NaH2PO4 1.25, NaHCO3 26, MgSO4 2, CaCl2 2, D-glucose 10, equilibrated to pH 7.4 with a gas mixture of 95% O2 and 5% CO2. Then one slice was transferred to the recording chamber (submerged type, FST, Inc.) in which it was superfused continuously at a rate of 2 ml/min with the ACSF maintained at 34°C. Extracellular recordings were performed using micropipettes (5–10 MOhm) filled with 4–M potassium acetate. A bipolar electrode (SNE-100, Rhodes Medical Institute, Inc.) provided the stimuli. Only one cortical and/or one hippocampal slice per animal was taken for electrophysiological recordings. In order to facilitate offline analysis of the recorded signals by improving the signal-to-noise ratio, the average of 2 to 6 responses at each time point was stored.

**Visual cortex.** A block of tissue containing the visual cortex was dissected and fixed on the cutting platform of the vibratome. Four hundred-μm thick coronal sections were cut between bregma–5.8 and –7.8 mm (according to Zilles 1985) with an inclination of 18° to the frontal plane. Extracellular field potentials (FPs) were recorded in layers II/III (300–350 μm below the cortical surface) of visual area OC1. The recording electrode was placed about 100-μm medial to a line perpendicular to the cortical surface and passing through the stimulation site in order to reduce the antidromic response. Evoked responses were elicited by stimulating layer IV. Stimulus pulses of 100-μs duration were applied at 0.033 Hz during test periods. The stimulus intensity was adjusted to evoke FPs of about 50% of their maximal amplitude and was not changed during high-frequency stimulation (HFS). Baseline recordings were performed until stable responses could be measured for about 30 min. HFS was applied, consisting of 5 theta bursts (10 bursts of 4 pulses at 100 Hz in 200-ms intervals) every 10 s. Following HFS, testing with single pulses was continued for 60 min. In Experiment 1, then paired-pulse stimulation was applied in the same slice with 7 different inter-stimulus intervals (ISIs) ranging from 20 ms to 5 s. The orthodromically evoked component of the response, with a peak latency of about 4–7 ms after the stimulus onset, was used for analysis. The amplitude of the FP was evaluated by measuring the voltage difference between the negative and the following positive peak of the FP (see Fig. 1).

**Hippocampus.** The hippocampus was dissected free and 450-μm transverse slices were cut. The stratum radiatum fibers proximal to region CA3 were stimulated electrically by pulses of 100 μs duration at 0.1 Hz. Extracellular recordings were obtained from the pyramidal cell body layer of the CA1 region. The stimulus intensity was adjusted to produce a population spike (PS) of about 40% of its maximal amplitude. Baseline responses were recorded for 30 min. LTP was induced using a theta burst stimulation consisting of 10 high-frequency bursts (4 pulses at 100 Hz) delivered in 200 ms intervals. Testing with single pulses was continued for 60 min after the application of HFS. The PS amplitude was evaluated by raising a perpendicular from the tangent line between PS onset and offset to the negative peak of the PS.

**Receptor-binding assays.** Membrane preparations from the occipital cortex and hippocampus of adult offspring from Experiment 1 were prepared, using a modification of the methods of Goodnough and Hoekstra (1995). The tissue was thawed on ice in 10 volumes of 0.32 M sucrose and homogenized with a TH tissue homogenizer (Omni International, Warrenton, VA). The homogenate was centrifuged at room temperature at 500 g for 20 min to remove cellular debris, and the supernatant was centrifuged at 35,000 g for 30 min. The resulting pellet was resuspended in an equal volume of distilled H2O to lyse any intact cells, and was centrifuged at 500 g for 10 min. The
supernatant was then centrifuged at 35,000 g for 30 min to obtain the membrane pellet. The membranes were washed one time in distilled H2O and twice in assay buffer (40 mM KH2PO4, 100 mM KCl; pH 7.4) at room temperature by centrifugation at 35,000 g for 30 min. After the last centrifugation, the pellet was resuspended in 10 volumes of assay buffer and stored at −80°C until use.

Binding of [3H]MK-801 (22.0 Ci/mmol, NEN Life Science Products, Boston, MA) to NMDA receptors was based on the method of Ransom and Stec (1988). Assays were performed at room temperature in an incubation volume of 1.0 ml in the presence of 50 µM glutamate, 50 µM glycine, and approximately 200 µg of protein per tube. For saturation binding experiments, 0.2−27 nM [3H]MK-801 was used. For single-point binding experiments, 10 nM [3H]MK-801 was used. Nonspecific binding was determined using 100 µM MK-801. Following a 1-h incubation period binding was terminated by rapid filtration and washing, using a Skatron cell harvester (Lier, Norway).

Binding of [3H]muscimol (19.1 Ci/mmol, NEN Life Science Products, Boston, MA) to GABA receptors was based on the method of Goodnough and Hawkinson (1995). Assays were performed at 4°C in an incubation volume of 0.5 ml with approximately 100 µg of protein per tube. For saturation binding experiments, 0.8−52 nM [3H]muscimol was used. For single-point binding experiments, 52 nM [3H]muscimol was used. Nonspecific binding was determined using 100 µM GABA. Incubations were for 1 h in the dark at 4°C, and were terminated by rapid filtration and washing.

Radioactivity on the filters was determined by liquid scintillation counting in plastic vials with 4 ml of Ultima Gold scintillation cocktail. Specific binding was calculated as the difference between total binding and nonspecific binding. Protein concentrations were determined according to the method of Bradford (1976). Values for Kd and Bmax were determined from saturation binding experiments, after fitting curves by nonlinear regression analysis using the PRISM program (GraphPad Software Inc., San Diego, CA).

**Brain PCB analysis.** PCB concentrations were determined in whole brains (n = 3 per exposure group) of female littersmates at weaning (PND 21) in Experiment 1, and at birth (PND 0) in Experiment 2 by gas chromatography with electron-capture detection (GC-ECD system: HRGC Mega 2, Fisons, Mainz, Germany). Briefly, after thawing, the brain samples were ground with a potter homogenizer (B. Braun, Melsungen, Germany). The homogenates were vortexed with formic acid in a test tube. Afterwards, PCBs were extracted by solvent extraction with n-heptane from the tissue-formic acid mixtures. The n-heptane extracts were purified by silica-gel chromatography using petroleum ether as the mobile phase. The gas chromatographic separation of the PCBs was carried out on 2 capillary columns of different polarity (DBS and DB1701, J & W Scientific, Köln, Germany). The 14 PCB congeners used to create the RM, and additionally PCB 52, were measured. Determination of PCB 52 was included, since it is always measured in routine analysis by the laboratory. The commercial mixture A1254 consists of more than 100 PCB congeners, whereby the concentrations of the 15 measured congeners amounted to about 50% of the total PCB concentration in the A1254 diet (Frame et al., 1996).

PCB detection limits lay within 0.005 and 0.01 µg/g (wet weight). PCB concentration in dams and offspring was expressed as the percentage of the PCB concentration in the A1254 diet that was passed to each of the dams during lactation. No overt signs of gross toxicity could be detected in dams or offspring.

**Experiment 1: Adult Animals**

PCB exposure levels in brains from offspring at weaning (PND 21) were 8.2 ± 2.4 µg/g wet weight for the RM-40 mg group and 1.0 ± 0.3 µg/g wet weight for the A1254 group, respectively, while for the control group, the values were below the detection limit. It must be noted that the PCB concentrations measured in the A1254 group were artificially low, which is partly due to congeners present in the A1254 mixture that have not been included in the analysis. Furthermore, 100% of the congeners in the RM mixture are resistant to metabolism, while only about 50% of the congeners in the A1254 mixture share this property. On the day of testing, the weight of the rats differed significantly among groups (males: controls, 441 ± 7.5 g; RM group, 389 ± 12.3 g; A1254-group, 348 ± 11.8 g; n = 12 per group, p < 0.0001; females: controls, 252 ± 3.5 g; RM group, 236 ± 3.6 g; A1254-group, 225 ± 3.5 g; n = 10 per group, p < 0.0001).

**Electrophysiological measurements.** The baseline recordings in layers II/III of cortical slices revealed no differences among the 3 groups with respect to the mean FP amplitude before HFS (control group: 0.49 ± 0.03 mV; RM-40 mg group: 0.42 ± 0.04 mV; A1254 group: 0.48 ± 0.04 mV; p = 0.391). Similarly, no differences were found with respect to stimulus intensity (control group: 0.36 ± 0.09 mA; RM-40 mg group: 0.24 ± 0.02 mA; A1254 group: 0.26 ± 0.03 mA; p = 0.857). Figure 1A shows examples of original traces of the evoked FP before and after HFS measured in slices of a control rat, a 40-mg RM-exposed rat, as well as a A1254-exposed rat. In Figures 1B and 1C, the LTP data from all animals of the 3 groups are summarized for female and male rats separately. The mean amplitude changes in relation to the baseline values (100% at time 0 ms) are indicated for 7 selected time points after the HFS.

Three-way repeated measures ANOVA revealed a marginally significant effect of treatment (F(2,61) = 2.82; p < 0.068). If the control group is compared only to the A1254 group, the effect of treatment becomes significant (F(1,41) = 5.22; p < 0.028), while a comparison of the control and the RM groups shows a marginally significant effect of the treatment (F(1,40) = 3.53; p < 0.068). Furthermore, a significant effect of gender on the amount of LTP was found (F(1,61) = 5.85; p < 0.019). Cortical potentiation was lower in females than in males. Thus, we analysed the data from males and females separately in addition. For the females, the overall effect of exposure is not significant, while a marginally significant effect is seen for the interaction of time and exposure (F(12,162) = 1.65, p < 0.084). If the controls are compared only to the A1254 group, this interaction is not statistically significant, while a comparison of the control to the RM 40-mg group reveals a statistically significant interaction (F(6,108) = 2.65,
p < 0.020). Single time periods after HFS that are statistically significantly different are indicated in Figure 1B.

For the males, no statistically significant overall effect of exposure or of the interaction of exposure and time was found. If only the controls are compared to the A1254 exposed rats, the effect of exposure became significant (F(1, 23) = 4.95, p = 0.036), while a comparison of the control and the RM-40 mg group showed no significant effects. For comparison with the females, single time periods after HFS that are statistically significantly different between exposure groups are indicated in Figure 1C.

Results from the paired-pulse procedure revealed that the FP amplitude evoked by the second stimulus could be either increased, unchanged, or decreased in relation to the first evoked response, depending on the interstimulus interval (ISI). The responses to paired-pulse stimulation were not affected by exposure to any of the PCB mixtures. The data for the 2 sexes were analyzed together, because no differences could be found between male and female rats. Figure 2 gives a summary of the paired-pulse data at all ISIs for the 3 groups, for males and females together. No statistically significant differences were found between the 3 exposure groups for the seven ISIs applied.

Binding studies. Saturation binding assays were used to determine receptor affinity (K_d) and receptor number (B_max) for NMDA and GABA-A receptors in the occipital cortex (Table 1). One-way ANOVA showed a statistically significant effect of exposure on B_max for NMDA receptors measured in the cortical preparations (F(2,28) = 6.555, p < 0.005). The number of [3H]MK-801 binding sites was decreased significantly by 41.9% in the occipital cortex of rats exposed to the RM-40 mg relative to control values. There was no statistically significant effect on [3H]muscimol binding in the occipital cortex (F(2,30) = 0.194, p = 0.825).

Because the cortical data showed no significant effect on receptor affinity, single point-binding assays were used in the succeeding analysis of the hippocampus in order to compare the number of NMDA and GABA-A receptors in the control, RM-40 mg, and A1254 groups. There were no statistically significant differences in [3H]MK-801 binding (F(2,27) = 0.623, p = 0.545) or [3H]muscimol binding (F(2,27) = 0.116, p = 0.891) between the treatment groups.

Experiment 2: Developing Animals

PCB exposure levels in brains from offspring at birth (PND 0) were 0.44 μg/g wet weight for the RM-5 mg group and 4.63 μg/g wet weight for the RM-40 mg group, respectively, while for the control group, the values were below the detection limit. No overt signs of toxicity were found in the pups: the PCB

![Graph](image-url)
concentrations applied did not lead to significant body-weight reductions when measured on the day of sacrifice (controls: 29.43 ± 1.46 g, n = 9; RM-5 mg: 27.71 ± 1.46 g, n = 9; RM-40 mg: 26.00 ± 1.58 g, n = 10; p = 0.294) nor to any other impairments of physical development.

Electrophysiological measurements: Visual cortex. The extracellular baseline recordings in layers II/III, evoked by layer IV stimulation, revealed no differences among the 3 groups with respect to the mean FP amplitude before HFS (controls: 0.32 ± 0.05 mV; RM-5 mg: 0.29 ± 0.03 mV; RM-40 mg: 0.27 ± 0.04 mV; p = 0.433). Likewise, the stimulus intensity applied did not differ between groups (controls: 0.23 ± 0.04 mA; RM-5 mg: 0.20 ± 0.03 mA; RM-40 mg: 0.17 ± 0.01 mA; p = 0.405). After the application of HFS, however, the effects of PCB exposure were apparent. While LTP could be established in cortical slices from control rats with a mean amplitude increase in relation to baseline measured 60 min after HFS to 122.63 ± 9.88%, the mean increase in slices from the RM-5 mg group amounted to only 113.86 ± 9.64%, and no increase at all is seen for the RM-40 mg group (98.22 ± 6.08%). As is shown in Figure 3A, the FP amplitude was potentiated after HFS in the control rat, whereas in the 2 PCB-exposed rats, no such clear increase was found. In Figure 3B, the LTP data from all animals of the 3 groups are summarized. The mean amplitude changes in relation to the baseline values (100%) are indicated for 9 selected time periods after the HFS. The initial decrease of the amplitudes immediately following the end of the HFS is seen in all 3 groups. Approximately 15 min after HFS, a stable increase can be seen for the control rats, while no such increase is found for the RM-40 mg rats and only a small increase for the RM-5 mg rats during the remaining period of 60 min.

The repeated-measures ANOVA revealed no overall effect of exposure, while a statistically significant effect on the interaction of time and exposure was found (F(16,168) = 1.87, p < 0.026). While a comparison of the control and the RM-5 mg group showed no significant effect, there was a marked, statistically significant effect on the interaction of time and exposure if the controls were compared to the RM-40 mg group (F(8,120) = 3.10; p = 0.003).

Hippocampus. The mean PS amplitudes in the 3 groups during baseline recordings were 0.56 ± 0.08 mV in the controls, 0.59 ± 0.08 mV in the RM-5 mg group and 0.47 ± 0.07 mV in the RM-40 mg group (p = 0.553). Likewise, the stimulus intensity applied did not differ between groups (controls: 0.48 ± 0.13 mA; RM-5 mg: 0.65 ± 0.11 mA; RM-40 mg: 0.51 ± 0.13 mA; p = 0.612). Figure 4A shows examples of original traces of the PS before and after the HFS for a control rat, an RM-5 mg rat and an RM-40 mg rat, respectively. All 3 animals show a clear increase in the amplitude after HFS. In Figure 4B, the LTP data measured in the CA1 region are summarized for all exposure groups. No statistically significant differences among the 3 groups were found. When performing repeated-measures ANOVA, neither the effect of exposure (F(2,25) = 0.08, p < 0.924) nor the interaction of time and exposure (F(16,200) = 0.88, p < 0.587) were significant.

DISCUSSION

Our results from Experiments 1 and 2 show that LTP in the occipital cortex is altered by a PCB mixture reconstituted according to the pattern found in human breast milk (RM) if the exposure takes place during embryonic and/or early postnatal development. This effect appears to be persistent since cortical LTP of adult animals is still affected, even though they were not exposed to PCBs after weaning. Based on our previous studies, PCB tissue levels would not likely differ from controls at this time (Lilienthal and Winneke, 1991).

In Experiment 1, adult female rats were affected by exposure to the RM, while adult male rats showed LTP deficits follow-
which showed reduced LTP, but not in a brain area (hippocampus) in which we have previously shown no effect on LTP in adult offspring after maternal exposure to single PCB congeners (Altmann et al., 1995). Furthermore, according to Experiment 2, in rat pups exposed to the RM cortical, but not hippocampal, LTP was affected, suggesting that similar results for hippocampal LTP could be expected in the adult rats. However, the relationship between decreases in [H]MK-801 binding and LTP is not clear, since cortical LTP was reduced in male offspring of the A1254 group in the absence of an effect on binding. In the female offspring, exposure to the RM reduced cortical LTP. Unfortunately, due to technical limitations we could not measure [H]MK-801 binding in the cortex of females. The lack of effect of [H]MK-801 binding in the A1254 group might partly be explained by the lower concentrations of congeners resistant to metabolism in the A1254 diet as compared to the RM diet. One might speculate that with respect to this parameter, the main effects are due to these metabolically resistant congeners. In addition, the PCB effects on NMDA receptors are assumed to be only one of several possible mechanisms leading to an impairment of LTP. The lack of effect of the exposure conditions on cortical paired-pulse inhibition as well as on cortical and hippocampal [H]muscimol binding sites in the 3 exposure groups suggests that the inhibitory system is not a target for PCBs.

In the adult offspring, a 10 to 20% reduction in body weight compared to controls was detectable in the RM group as well as in the A1254 group in both sexes. This effect of maternal PCB exposure on body weight was seen in previous studies using rats (e.g., Hany et al., 1999a). In the latter study, however, reduced body weight was accompanied by an increased brain weight of the exposed animals. It remains to be investigated in future studies whether the decrease in body weight is related to impairment of LTP. In previous studies from our lab, PCB-induced LTP deficits were found in the absence of any body weight differences (Altmann et al., 1995). Furthermore, in Experiment 2, LTP was impaired in the cortex of rat pups following PCB exposure in the absence of a statistically significant effect on body weight.

In Experiment 2, the expression of LTP in the developing visual cortex was found to be more susceptible to RM exposure than LTP in the developing hippocampus. No statistically significant PCB-related alterations of the magnitude of LTP were found in hippocampal CA1. This confirms previous studies showing that developmental exposure to single PCB congeners reduced cortical, but not hippocampal LTP (Altmann et al., 1995, 1998).

In contrast to our findings in hippocampal CA1 following exposure to the reconstituted PCB mixture, it was reported recently that maternal exposure to the commercial mixture A1254 reduced LTP in the hippocampal dentate gyrus of adult offspring in vivo (Gilbert and Crofton, 1999; Gilbert et al., 2000). A number of differences in the 2 studies might account for these outcomes. Most importantly, LTP was measured in 2 hippocampal subfields with several marked differences: Hört-
nagl et al. (1991) reported that the levels of noradrenaline, GABA, and glutamate decarboxylase activity were highest in the dentate gyrus and lowest in CA1, while the concentration of somatostatin was highest in CA1. Goldschmidt and Steward (1980) found that colchicine lesions selectively destroyed dentate granule cells while sparing CA1 pyramidal cells. Differing effects were measured for the action of noradrenaline on CA1 in comparison to dentate gyrus neurons (Madison and Nicoll, 1982). The investigation of the developmental profile of LTP in the 2 regions revealed that LTP reaches adult values about 2 weeks after birth in CA1 but only about 3 weeks after birth in dentate gyrus (Bekenstein and Lothman, 1991). In addition, the electrophysiological methods for in vivo vs. ex vivo recording are different. Another important factor might have been the exposure protocol. Gilbert and Crofton (1999) and Gilbert et al. (2000) exposed dams to A1254 via oral gavage from gestational day 6 to PND 21. In the present study, exposure of the dams via food started long before mating and was stopped at birth. It is possible that the dosing regimen used by Gilbert and Crofton (1999) and by Gilbert et al. (2000) could have resulted in higher brain PCB concentrations during development, although brain PCB concentrations were not reported in these papers. Finally, the effects found on LTP in the hippocampal dentate gyrus might be related to indirect effects of PCB exposure on functions of the amygdala, since LTP in dentate gyrus was found to be reduced in amygdala-lesioned rats (Ikegaya et al., 1994). A direct comparison of LTP in the 2 hippocampal subfields following identical exposure conditions remains to be done in future studies, in order to evaluate more clearly the role of the hippocampus in PCB neurotoxicity.

The present findings correlate with rat behavioral studies from our lab, where deficits were found following developmental exposure to PCBs in tasks depending primarily on extra-hippocampal function, namely the catalepsy test, as well as the test for passive avoidance behavior (Weinand-Hä rer et al., 1997), while no deficits were found using the Morris water maze and the radial arm maze (Lilienthal et al., 1997), 2 hippocampal-dependent tasks. Similarly, no deficits in water-maze learning were detected in A1254-exposed rats (Gilbert et al., 2000). This is in accordance with results of gestational treatment with single PCB congeners which failed to alter learning and memory in the radial arm maze, but induced deficits in delayed spatial alternation, a task known to depend on the function of the medial prefrontal cortex (Schantz et al., 1995). Several studies found deficits in cortical function following prenatal PCB exposure of rats (review in Brouwer et al., 1995) as well as humans (Jacobson et al., 1985, 1990). Contrary to these findings, impaired radial-arm-maze performance was reported recently in A1254-exposed male rats (Roeggé et al., 2000) suggesting a participation of the hippocampus in neurotoxic effects of this PCB mixture.

Because LTP deficits after maternal exposure were not only observed in immature but also in adult offspring at a time when PCB levels in the brain had returned to background levels, the effects are likely due to changes in developmental processes. Several studies using prenatal PCB exposure have reported changes in the level of biogenic amines and hormones as well as effects on neurotransmitter systems (overview in Brouwer et al., 1995; Seegal, 1996). The deficits in LTP measured in our animals may be due in part to these neurochemical effects during development. It has been suggested that neurochemical effects may also underlie PCB-related changes in memory and learning observed in rodents (e.g., Lilienthal et al., 1990). The PCBs exert pro- or anti-estrogenic effects, depending on the test system and the congeners under study (Hany et al., 1999b; Safe et al., 1991). As steroids like estradiol are known to be involved in neuronal plasticity and are important for CNS development (McEwen, 1992), changes in the brain hormone levels and/or in brain hormone receptor binding are possible mechanisms mediating the neurodevelopmental effects of PCBs. In addition, Morse et al. (1996) found that the perinatal exposure to PCB decreased the level of brain thyroid hormones. Alterations in the thyroid function have been shown to affect development of biogenic amine neurotransmitters, behavior, as well as LTP (Pavlidès et al., 1991).

In summary, the maternal exposure of rats to PCB mixtures resulted in a reduction of LTP in visual cortical slices of the exposed animals when compared to controls, as well as in a reduced number of [3H]MK-801 binding sites in the occipital cortex. There were no PCB-related alterations in hippocampal CA1. The effects on LTP were observed not only in rat pups but also in adult rats long after the cessation of the PCB treatment. Our results obtained in visual cortical slices correlate with behavioral studies in animals and humans, which showed that deficits in learning and memory tasks were produced by in utero exposure to PCBs and persisted through adulthood long after the end of exposure. We have demonstrated here that the immature, as well as the mature, occipital cortex is functionally altered by a PCB mixture composed of the congener spectrum found in human breast milk at brain levels of the exposed offspring that lay within 1–2 orders of magnitude above background concentrations in humans.

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