Gestational exposure to 2,2',4,4',5,5'-hexachlorobiphenyl(PCB153) impairs learning performance in rat offspring

Rieko Hojo[1], Masaki Kakeyama[1], Junzo Yonemoto[1], Chiharu Tohyama[2]

[1]Institute for Environmental Studies
[2]The University of Tokyo

Several epidemiological studies reported negative associations between prenatal polychlorinated biphenyl (PCB) exposure and measures of cognitive functions in infancy or childhood, but a limited amount of data hamper to elucidate congener-specific analysis on the neurodevelopmental adverse effects[1]. Although some studies showed that gestational and lactational exposure to PCB congeners impaired learning performance in monkeys[2] and rats[3], it is largely unknown which congeners exert neurotoxicity and when the most vulnerable period is during development. In the present study, focusing upon 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153), the most abundant congener of PCBs in human body[4], we studied (1) whether there is any critical window of exposure during gestation and lactation, (2) how PCB153 affects learning performance with the schedule-controlled operant behavior (SCOB).

Materials and Methods

Long-Evans Hooded rats were divided into 5 groups (6-8 per each group), and administered PCB153 at a single oral dose of 2.0 mg/kg bw. Rats were administered either on gestational day (GD) 5, 15, postnatal day (PND) 3, or on GD 5, 15 and PND 3 (GD5&15&PND3group). Control rats received corn oil containing 0.5% nonane on GD 5, 15 and PND 3. On 9-11 weeks of age, one male pup from each dam was randomly selected for the behavioral experiment. The learning behavior was conducted in operant chamber boxes, containing two levers positioned along one wall. When rats made a correct response to the lever, they were provided with a 45 mg diet pellet. Scheduled control and data acquisition were accomplished by ComPACT ops/w operant behavioral test control system. In the SCOB, Fixed Ratio (FR) 20 schedule was conducted for 15 days (1 session/day and 5days/week). In FR20 schedule, the 20th lever pressing resulted in the reward of a pellet. The session was terminated when rats acquired the maximum number (50) of pellets or when a maximum time (30 min after the beginning of experiment) lapsed.

Difference in the mean was considered statistically significant at $p<0.05$. Data for dam's body weights, pup's body weights, number of pups per dam and the ratio of male and female pups per litter at birth among groups were analyzed by two-way analysis of variance (ANOVA). Other dose-response data including the number of animals that met the requirement for the learning achievement criteria (animals acquired more than 43 out of 50 pellets. i.e. 85% of the total pellet, in one session) and reward rates were analyzed by X^2 test and two-way ANOVA followed by the Scheffé's post-hoc test, respectively.

Results and Discussion

Body weights of dams and pups, the number of pups per dam and the ratio of male and female pups per litter at birth were not significantly different between the 4 experimental groups and the control group (data not shown).

In the control group, 4 out of 8 animals met the requirement for the learning achievement criteria in the first session, and all animals in this group satisfied this criteria from 7^{th} to the end of the test (15^{th} session), being significantly higher than that in the 1^{st} session (Fig. 1 (A)).

Exposure to PCB153 during the developmental period, except a group of rats exposed lactationally only (PND3 group), altered the learning and behavioral performance of offspring in the SCOB. That is, in the GD5, GD15 and GD5&15&PND3 groups, the number of rats that met the requirement for both the learning achievement criteria and reward rates were lower than those of control group throughout the test. Only 3 out of 6 animals in the GD5 group (in the 10^{th}, 11^{th} and 13^{th} session), 4 or 5 out of 8 animals in the GD15 group (in 7^{th} and 13^{th} session, respectively), and 2 to 4 out of 8 animals (from 7^{th} to 13^{th} session) in the GD5&15&PND3 group significantly satisfied the learning achievement criteria (Fig. 1). The reward rates in these groups (85.1 ± 1.2 for the GD5 group, 80.3 ± 1.9 for the GD15 group, 79.3 ± 0.1 for the GD5&15&PND5 group) were also significantly lower than that in control group (91.9 ± 0.6, Fig.2). In contrast to these gestational exposure groups, the PND3 group did not have a significant difference from the control group in terms of the number of animals that met the requirement for the learning achievement criteria. In addition, no significant difference in reward rate was observed between the PND3 group and the control group (Fig.2).

The present results showed that the learning behavior of pups, born to dams exposed to PCB153 during development was adversely affected. The characteristic features of the present results can be summarized two-fold. First, to our knowledge, a single oral dose of 2.0 mg/kg of PCB153 is the lowest dose so far tested to show clear-cut effects on learning and memory function. A Lowest Observed Adverse Effect Level (LOAEL) of PCB153 for a decrease in Long-Term Potentiation (LTP) in the hippocampus of rats[5] and the one for Aroclor 1254 for learning behavior of rats[6] was reported to be 50 mg/kg (a daily dose at 1.25 mg/kg from GD3 to PND21) and 210 mg/kg (a daily dose at 6 mg/kg from GD6 to PND21), respectively.

Second, effects of PCB153 on the learning behavior were clearly observed by exposure in the gestational period, but not by lactational period. In addition, the reward rate in the GD15 group was significantly lower than that of the GD5 group, suggesting that a learning deficit is more pronounced in the late gestational period. In contrast, a few preceding experimental studies reported that monkeys exposed to PCB mixtures via lactation only impaired the memory and learning function as determined by discrimination reversal and spatial delayed alternation[2], and that in rats, both gestational and lactational exposures to mixture of PCBs exert adverse effects on the learning performance[3]. Epidemiological Lake Michigan cohort studies reported that prenatal PCB exposure as determined by PCB concentrations in the umbilical cord blood, not the lactational exposure, was negatively correlated with greater impulsivity, poorer concentration, and poorer verbal, pictorial, and auditory working memory[5], which might be supported by the present result.

The ortho-substituted congeners are persistent in the human body and cause neurotoxic actions by Ah receptor-independent mechanisms[6]. It has been shown that both acute and chronic exposure to PCB153 causes a significant reduction in LTP in the rat hippocampus[6,7]. It has also been reported that the ortho-substituted congeners inhibit the brain second messenger systems such as Ca^{2+} buffering[8] and the brain dopaminergic system such as the uptake into synaptic vesicles[9] and the synaptosomal dopamine content[10]. Elucidation of mechanism and risk assessment of developmental neurotoxicity of non-coplanar PCBs including PCB 153 warrant a further study.

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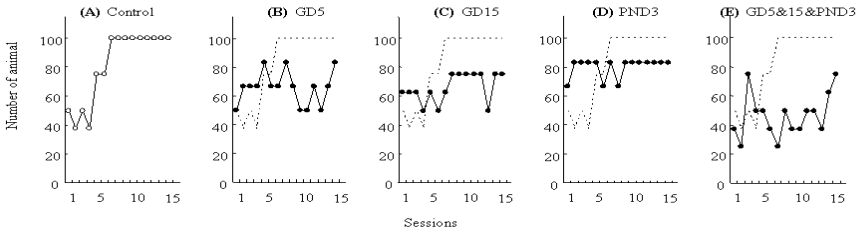

Figure 1. The number of animals met the requirement for the learning achievement criteria for 15 sessions in FR 20 schedule. (A) Vehicle-exposed control; (B-D PCB153 was administered on specified day with a single oral dose of 2 mg PCB153/kg. Broken lines in panel (B), (C), (D) and (E) are the data of control group depicted for a reference purpose.

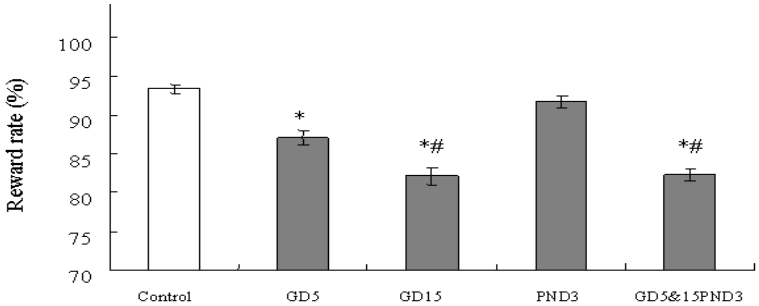

Figure 2. Reward rates (number of food pellet/minute) during FR20 schedule. See the Legend to Figure 1. The data is expressed for means ± S.E.M. for 6-8 rats. * indicates $p < 0.05$, vs. Control and # indicates $p<0.05$, vs. GD5.