MATERNAL EXPOSURE TO A LOW DOSE DIOXIN AFFECTS THE PAIRED ASSOCIATIVE LEARNING BEHAVIOR OF OFFSPRING OF LONG-EVANS RATS

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Abstract
We studied effects of maternal exposure to a low dose of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) or 2,3,7,8-tetrabromo-dibenzo-*p*-dioxin (TBDD) on the paired-associative learning behavior in rat offspring. Pregnant Long-Evans rats were dosed by gavage with 0, 200 or 800 ng/kg of TCDD or TBDD on gestational day 15. First, *in utero* and lactational exposure to TCDD at 800 ng/kg caused a 10-15% body weight loss from postnatal day (PND) 91 and thereafter. A similar trend was observed for TBDD at 800 ng/kg. These results demonstrate the manifestation of action of these compounds exposed *in utero* and via lactation later in adulthood. In the behavioral test, the acquisition of paired-associative memory was examined by a sophisticated learning test methodology, named Flavor Map test, using an event arena apparatus. We found the anxiety-like behavior at the time of habituation in animals born to dams administered TCDD or TBDD at 200 ng/kg. These animals showed significantly longer latency than control animals. Thus, TBDD was found to have TCDD-like effects on body weight and an anxiety-like symptom. In the Flavor Map testing, maternal exposure to 200 ng TCDD/kg was found to suppress the acquisition of paired-associative memory of offspring, demonstrating the perturbation of this advanced learning ability by a very low TCDD dose.

Introduction

Maternal exposure to dioxins has been reported to affect the advanced brain function of offspring even when the exposure level is too low to affect their mothers. On a molecular level, a single oral dose of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) of 200 or 800 ng/kg on gestation day (GD) 15 did not affect dams of Long-Evans strain, but it altered mRNA levels of glutamate NMDA receptor that has a critical role in learning and memory function in the neocortex and the hippocampus[1]. On a behavioral level, a similar maternal dose of TCDD affected the learning performance of offspring in the schedule-controlled operant behavioral task, especially in the multiple schedule of FR and DRL tasks, whereas no effect on the FR or DRL only[2]. These results suggest that TCDD appears to affect complicated learning tasks rather than simple ones.

Recently, we established and reported a novel sophisticated learning test methodology in rats, named 'Flavor Map testing', which enabled to examine the paired-associative learning performance of rats using an event arena apparatus[3]. In the Flavor Map test, animals were subjected to learn six flavor-place associations in the event arena within a few weeks. To our knowledge, it is the first behavioral task that animals use schema to refer to preexisting knowledge structures, and the most complicated learning task for the rat so far reported. Thus, the Flavor Map test could be a very useful methodology to clarify the quality of neurotoxicity of chemicals on the advanced brain function.

Here, we examined effects of maternal exposure to TCDD on the paired-associative memory function of rat offspring by using the Flavor Map test on the event arena. In addition, effects of maternal exposure to

2,3,7,8-tetrabromo-dibenzo-*p*-dioxin (TBDD) were also examined, because polybrominated dibenzo-*p*-dioxins (PBDDs) have AhR agonist properties and cause dioxin-like effects, and more REP studies on PBDDs are urgently needed[4].

Materials and Methods

Animals and exposure: Pregnant Long-Evans Hooded rats were dosed by gavage (2.5 ml/kg) with 0 (vehicle), 200, or 800 ng TCDD or TBDD/kg (5-6 animals in each exposed group). Two day after birth, five males and five females were culled from the litters to allow for similar lactational TCDD exposure. Offspring were weaned on postnatal day (PND) 21 and unisexually group-housed (five pups). On PND 80, one male offspring per litter (5-6 animals in each exposed group) was randomly selected for the behavioral experiment, and maintained at 85% of free-feeding weight. The other male offspring per litter was monitored for body weight gain per week under a free-feeding condition throughout the test. Animal experiments and handling of hazardous chemicals were performed, according to the guidelines for animal experiments and environmental safety, respectively, at the University of Tokyo.

Behavioral test: The event arena and the Flavor Map testing were described previousely[3]. Briefly, The event arena (1.6 m by 1.6 m) contains a 7 × 7 grid of locations at which sand wells can be made available and four surrounding start boxes. (Fig. 1A). Sand-wells of a special design to help mask the flavored food hidden within the sand, could be placed into these holes. The animals had to dig through the sand to secure each food pellet. After habituation to the event arena and sand-wells, animals were trained learn 6 flavor-place associations concurrently, using different flavors of food (flavor cues) and sand-wells (place cues) located within the event arena (Fig. 1B). After being given a cue flavor in a start box, the animals recall the spatial location with which it is associated, and run into the arena to that location to secure more of that flavor of food. The animals visited and sometimes dug at incorrect sand-wells, which did not contain food on that particular trial, until they found the correct one. On each trial, the animals would retrieve the first of three buried food pellets, return to the start box to eat it, and then run back to the correct sand-well to collect and transport the second and third pellets. One hour later, the second trial began with a different cue flavor in the start box and a different sand-well baited.

Statistical Analyses: All statistical analyses were carried out by using the SPSS 15.0 data analysis with comprehensive statistics software (SPSS Japan Inc, Tokyo). A difference was considered significant at $p<0.05$. Data for dam's body weights, pup's weights and the number of pups per dam were analyzed by two-way analysis of variance (ANOVA) with repeated measurements. Other dose-response data were analyzed by one-way or independently two-way ANOVA, followed by Scheffé's post-hoc test.

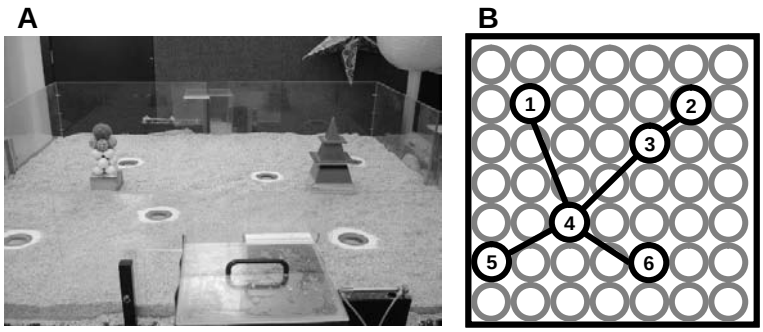

Fig. 1. Paradigm for Flavor Map testing. (A) The event arena and four surrounding start boxes. (B) The spatial arrangement of the six flavor- place pair and the "schema" this constitutes: 1; Chocolate, 2; Cherry, 3; Anise, 4; Bacon, 5; Coconut, 6; Strawberry.

Results and Discussion

TCDD and TBDD decreased offspring body weight later in adulthood

TCDD exposure in this experiment did not affect maternal body weight gain during gestation period (21 days of all animals) and viability of dams and pups (no animals died throughout the test). All litters had 14–17 offspring, with no significant difference among groups. However, exposure to 800 ng TCDD/kg caused a 10-15% body weight loss from PND 91 to the end of the test, whereas there was no significant difference from PND 1 to 84, which supports the previous observations in which the same TCDD dose regimen significantly inhibited body weight gain after PND 49[5]. Thus, these inhibitory effects on body weight gain are thought to be a typical TCDD-like effect. In addition, exposure to 800 ng TBDD/kg also caused a significant body weight loss from PND 105 to PND 259. These results indicate that maternal exposure to TBDD also disrupt body weight of offspring in adulthood similar to the manner observed by TCDD exposure.

TCDD and TBDD induced anxiety-like behavior in the event arenas

Habituation for the event arena apparatus consisted of a series of stages across sessions, allowing exploration of the arena and its cues, experiencing each of the 4 start-boxes, digging for food, and carrying pellets to the start-boxes to eat. During habituation, TCDD as well as TBDD significantly increased the latency to enter the stage from a start box in a dose-specific fashion. In control animals, it took approximately 30 seconds for first 3 days, and then reduced to 10 seconds on the 5th day (Table 1). Almost the same trend of a decrease in latency was found in TCDD- and TBDD- exposed animals. However, 200 ng TCDD/kg-exposed and 200 ng TBDD/kg-exposed animals showed significantly longer latency than control animals from 3rd to 18th day of habituation (Table 1). These results strongly suggest that maternal exposure to 200 ng/kg of TCDD and TBDD dose-specifically induced an anxiety-like behavior under learning task. To our knowledge, this is the first to indicate that dioxin can induce an anxiety-like behavior. These results also indicate that TBDD causes a TCDD-like effect on affective disorder like symptom.

TCDD perturbed the paired-associative learning performance

In the Flavor Map test, maternal exposure to a low dose of TCDD was found to suppress the acquisition of paired-associative memory of offspring. To investigate the properties of paired-associative learning and its consolidation, we examined acquisition of sand-well choice behavior during training, and a “performance index” was calculated (Fig. 2). The performance of control animals was gradually increased across the sessions and significantly above chance from session 26 ($p<0.05$), as same as previous report[3]. Dioxin-exposed animals showed almost the same performance as controls, except for 200 ng TCDD/kg-exposed group. In 200 ng

Table 1. Anxiety-like behavior by maternal exposure to TCDD and TBDD.

	0	TCDD		TBDD	
		200	800	200	800
Day 5	9.5±1.74	36.5±5.03*	15.0±3.59	63.3±5.53*	31.8±4.48*
Day 10	12.0±2.18	30.5±2.85*	14.8±0.73	28.0±6.67*	12.3±1.20
Day 15	10.0±3.60	45.3±7.94*	7.3±1.45	26.2±9.42*	10.7±2.13
Day 20	3.3±0.93	6.8±2.26	3.8±0.84	5.7±2.41	6.5±1.93

Data indicates latency to enter the stage during habituation of the event arena learning behavior task. 0; vehicle-exposed controls, TCDD and TBDD; TCDD- and TBDD-exposed groups, respectively, 200 and 800; 200 ng/kg and 800 ng/kg exposed group, respectively. *indicates $p<0.05$, v.s. control group (n=4 in each group).

TCDD/kg-exposed group, performance index remained at the chance level throughout the test, being significantly lower than that in control group ($p<0.05$, Fig. 2). These results indicate that a low dose of TCDD suppressed acquisition of paired-associative memory of offspring, and that TBDD may have a distinct effect from TCDD effect on it.

Taken together, the present study demonstrated that exposure to 800 ng TCDD/kg reduced body weight of Long Evans offspring later in adulthood, and that exposure to 200 ng TCDD/kg induced anxiety-like behavior of offspring and perturbed a complicated learning ability. It also demonstrated that TBDD caused TCDD-like effect on body weight and anxiety-like symptom.

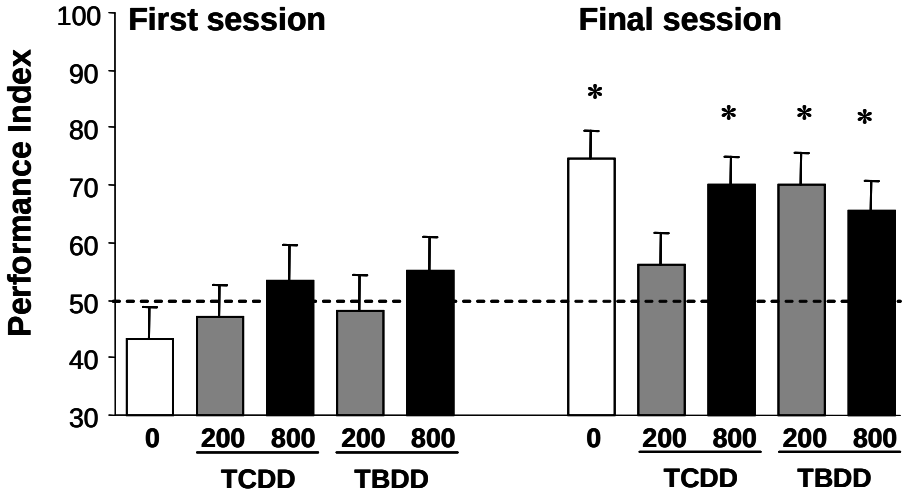

Fig. 2. Inhibition of acquisition of paired-associative memory by maternal exposure to TCDD. Performance index was computed as 100 – [100 × (errors/5)]. Control group showed significantly above chance value (=50.0, dotted line) on the last session. However, 200 ng TCDD/kg-exposed groups kept on chance value and significantly lower than that in controls. *indicates $p<0.05$, v.s. chance value.

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