EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN EXPOSURE DURING PREGNANCY ON THE NEURODEVELOPMENT OF RAT OFF-SPRINGS

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Introduction

The effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) during pregnancy have been reported to affect higher brain functions such as learning in adult off-springs. However, effects of prenatal exposure of TCDD on neural development as well as these brain functions in young off-springs remain unclear. In the present study, effects of TCDD on motor development and learning processes of rat off-springs were investigated in childhood and adulthood.

Materials and Methods

The study consisted of 3 experiments with fetus and off-springs exposed to TCDD. TCDD or corn oil was orally administered to pregnant rats during the 9^{th} - 19^{th} gestational day (GD). Both of exposed and control off-springs were fed with *ad libitum* access to maternal breast milk after birth, and to food and water after weaning.

Effects on the fetal brain

On the19th GD, fetus of 6 TCDD group and 5 controls, and placenta were removed from the uterus. The fetal brain and liver were collected from the fetuses, and the brain was divided into three parts: 1) the forebrain without cerebral cortex, 2) the cerebellum and brain stem, and 3) cerebral cortex.

Effects on neuro-development of off-springs

After birth, both the TCDD-exposed and control off-springs were fed *ad libitum* with free access to maternal breast milk.

From the 4th to 14th postnatal day (PD), motor development was investigated by measuring latencies to turning around completely when the pups were put upside down in an inclined plate. Active avoidance learning was accessed in a shuttle box using a conditioned tone associated with electric shock every day (10 trials/day) from the 31st to 44th PD in childhood.

In a different group of male off-springs, an active avoidance learning test in a shuttle box was also conducted when they became 19 weeks old. Five TCDD exposed and 6 control male rats were trained in a short schedule; 50 trials in one day. Other 10 TCDD exposed and 11 control male rats were trained in a long schedule; 10 trials per day for 5 days, a total of 50 trials.

Behaviors for the exposed and control groups were analyzed by 2-way ANOVA for repeated measures with the blocks of trials (4 or 3 levels) as factor.

Results and Discussion

1. *Effects on the fetal brain*

Mean weight of the whole brain of the TCDD exposed group (N=45) was significantly lower than that of the control group (N=54). However, the ratio of the brain to the whole body was larger in the exposed group, because the body weight of the exposed group was smaller than that of controls. When the brain was divided into three parts, the ratio of the forebrain without cerebral cortex to the whole brain in the TCDD exposed group was significantly larger than that of the control group (Table 1). This suggests that fetal growth, especially brain development is premature in the TCDD exposed group compared with the controls.

2. *Effects of TCDD on neurodevelopment of the off-springs*

2.1 Effects on off-springs in infancy

Figure 1 shows mean latencies across the 4 blocks of the trials (the 4-6th, 7-9th 10-12th, 13-14th PD) in turning around in the inclined plate. In both sexes, a main effect of TCDD/control was significant, as well as time effect, indicating that TCDD significantly delayed motor development of the off-springs. Since an interaction between TCDD/control x time was significant, subsidiary comparisons were conducted in each block; latency was significantly longer in the exposed group in the 3 blocks after the 7th PD in the male off-springs, and in the 7-9th and 10-12th PD in the female off-springs (Fig. 1). These results suggest that motor development of the TCDD exposed off-springs was delayed in early developmental age in both sexes.

2.2 Effects off-springs in childhood

Figure 2A shows latency of avoidance behavior of the male off-springs in a shuttle box across 3 blocks (the 31-35th, 36-40th, 41-44th PD). A main effect of TCDD/control for latency was significant, indicating that overall latency was significantly different between the TCDD exposed and control groups. Furthermore, spontaneous activity during the inter-trial interval was significantly lower in the TCDD exposed than control rats (Fig. 2 B).

These results indicated that learning was disturbed, and locomotor activity was reduced in the TCDD exposed off-springs, especially in male.

2.2 Effects on off-springs in adulthood

The avoidance rate was significantly lower in the TCDD exposed male off-springs than the controls in a short schedule for one day (Fig. 4). However, there was no significance difference between the TCDD exposed and control groups in the long schedule training for 5 days (data not shown). These results suggest that the limbic system involved in rapid learning might be deficient in the exposed group.

3. Conclusion

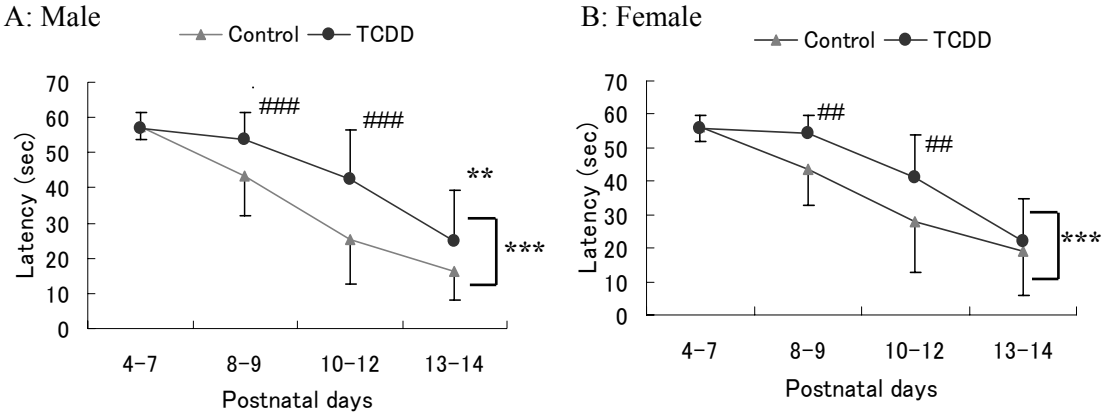
These results demonstrated that TCDD exposure during pregnancy affected neurodevelopment of off-springs during growth period and rapid learning in adulthood, especially in male rats.

Table 1 Comparisons of fetal brain growth between TCDD exposed and control groups

		Controls (N=54)		TCDD (N=45)		
		Mean	SD	Mean	SD	
Body weight	(g)	2.48	0.21	2.22	0.22	***
Brain	Weight (mg)	140.7	10.1	134.4	9.1	**
	/Body W (%)	5.7	0.48	6.1	0.41	***
Cerebral cortex	Weight (mg)	67.6	5.9	63.9	3.9	**
	/Body W (%)	2.7	0.21	2.6	0.89	
	/Brain W (%)	48.2	4.8	47.5	2.3	
Cerebellum & Brain stem	Weight (mg)	35.7	5.4	34.9	3.4	
	/Body W (%)	1.4	0.23	1.6	0.16	**
	/Brain W (%)	25.3	3.4	25.9	1.8	
Forebrain	Weight (mg)	19.9	2.8	20.8	2.1	
	/Body W (%)	0.81	0.14	0.94	0.9	***
	/Brain W (%)	14.2	2.1	15.5	1.1	**

W: weight

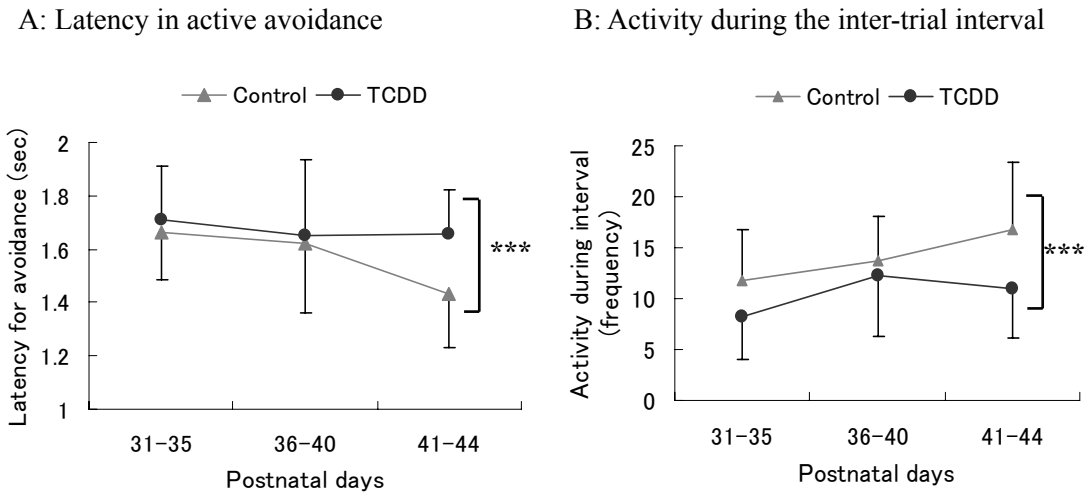
***:P<0.001, **:P<0.01 : Significant difference between the TCDD and control groups



***: P<0.001 significant difference for a main effect of TCDD/control

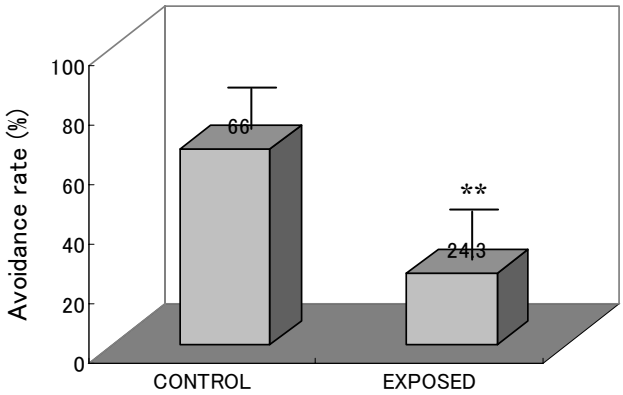
##: P<0.01, ###: P<0.001 significant difference between the TCDD and control in each block

Fig. 1 Comparisons of latency in an active avoidance test between the TCDD exposed and control off-springs from the 4th to 14th postnatal day in the male (A) and female (B) rats.



***: P<0.001 significant difference for a main effect of TCDD/control

Fig. 2 Comparisons of mean latency in an active avoidance test (A), and activity during the inter-trial interval (B) between the TCDD and control groups



**: P<0.05 Significant difference between 2 groups

Fig. 3 Comparisons of avoidance rate in a short schedule (50 trials in one day) between the male TCDD exposed (N=5) and control (N=6) off-springs aged 19 weeks.